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RELATIVE GROWTH IN THE NORTHERN FUR SEAL

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(Received for publication June 16, 1953)

PURPOSE

The first purpose of this paper is to describe the growth of the fur seal, *Callorhinus ursinus* L., as indicated by five measurements: body weight and length, hind flipper length, and skull length and width. A second purpose is to place on record for their practical value in the Alaskan fur seal industry selected measurements of known age fur seals from newborn to age ten years, together with measurements of older, full-grown seals of uncertain age.

The critical advice of Dr. D. G. Chapman, Laboratory of Statistical Research, University of Washington, is gratefully acknowledged.

METHODS AND DEFINITIONS

Freshly killed specimens were measured, most of them on St. Paul Island, Pribilof Islands, Alaska, during the midsummer breeding season. A few were examined at sea near Sitka, Alaska, during the winter, and a few at sea near Honshu, Japan, during the spring. The data from specimens taken off Japan are used in a single instance (hind flipper length of females). Wilke in 1951 and in a later unpublished study established the fact that Asian and Alaskan seals intermingle at sea and are anatomically indistinguishable.

Newborn young were selected on the evidence of the fresh umbilical cord. Seals of ages 1 to 10 years were sampled from several lots of marked, known-age animals. During the course of the present study, from 1940 to 1951, seal pups about 3 to 4 months old were marked by hot-iron brand or metal tag and marked seals were recovered later for study. Young males 2 to 5 years of age are best represented in the study collection because they were easily obtained during commercial sealing drives. Older animals are poorly represented because of the difficulties of removing them from the crowded breeding

grounds. Yearlings were sampled on the Pribilofs at the only season when they are available, in the fall, about two months after the close of the sealing season. The seals that return to land as yearlings arrive in late September and October and are probably the larger, more advanced members of their class. Thus, the measurements of yearling skulls are somewhat greater than one would expect (Figures 5 and 6).

Marked specimens were taken at random, with two exceptions. In 1944, tagged 3- and 4-year males were recovered from a commercial harvest of 40,000 animals, each selected on the basis of body length. That is, the sealing crew screened the driven animals for males of commercially useful size, 41 to 45 inches in length. Upon some of these screened animals the present estimates of skull size in the 3- and 4-year male are based (Tables 4 and 5). It seems unlikely that a serious error is thus introduced, judging from the observation that body sizes of selected and unselected animals are similar. That is,

	Selected by clubbers		Unselected by clubbers	
	N	Mm	N	Mm
Mean body length of 3-year males	21	1137	90	1149
Mean body length of 4-year males	22	1255	26	1257

Beyond age 10, male seals were sampled on the basis of field characters and were studied together as a ">10" group. It is easy to distinguish a bull, or fully adult male, by its large size, coarse mane, and long whiskers. Full-grown females were sampled, not by size but on the basis of tooth-ridge counts (Scheffer, 1950). The root of each tooth shows a ridge for each year of life up to about the tenth, when the ridges become obscure. The ridge represents a period of active deposition of dentin, presumably during the pelagic phase of the annual life cycle.

Where the growth curve is still rising beyond known-age 10, an asymptote has been placed by extrapolation at age 13 (Figures 2 to 6). This limit seems to fit the observed values for all but male skull measurements, to be discussed later. The estimated maximum life span of the seal is 30 years. The curves in Figures 2 to 6 were prepared by inspection.

The measurements used in the study are defined as follows:

Body weight; unbled in newborn seals, bled slightly in nearly all

older seals; fur dry whenever possible; to nearest quarter-pound or pound, depending upon the size of the seal.

Body length; a straight line from snout to tip of tail flesh; seal lying outstretched on its back; to nearest millimeter. The vertebral column of a seal, especially the cervical portion, is bent by rigor mortis and the observed variation in body length includes this error.

Hind flipper length; from center of curvature (tibiotarsal joint) of anterior angle of flipper, to tip of first digit. This digit is usually the longest one, although occasionally it is slightly shorter than the second.

Condylobasal length; (Figure 1) from transverse line touching most posterior points on occipital condyles to transverse line touching most anterior points on premaxillary bones. In the fur seal, this is the greatest length of the skull.

Mastoid width; (Figure 1) greatest transverse dimension of cranium across, or near, mastoid processes, outside of one to outside of the other. In all newborn fur seals this is the greatest width of the skull. By the end of the first year the zygomatic width has surpassed it.

Results

The measurements of five variates according to age and sex are given in Tables 1 to 5, with interpretations in Tables 6 and 8 and Figures 2 to 6.

Body weight. The young fur seal nurses for about three months, from approximately mid-July to mid-October. Weights of pups immediately after weaning are not available, although weights for 173 pups taken on 4 October are shown in Table 1. At this time the pups had grown to about two-and-one-half times their newborn weight. After weaning, the weight curve of the male is strongly sigmoid, with rapid growth in the seventh and eighth years of life. In the latter years sociological maturity is reached and the secondary sex characters develop. In the male the secondary sex characters are indicated by the great layer of subcutaneous fat which enables the breeding bull to fast for two months, the heavy neck and chest muscles used in fighting, and the mane or nape hair. Puberty in the male, as defined by the production of sperm, occurs during the fourth or fifth years, or rarely, the third year. It is noteworthy that the most rapid increase in weight of the male (excluding the nursing period) occurs

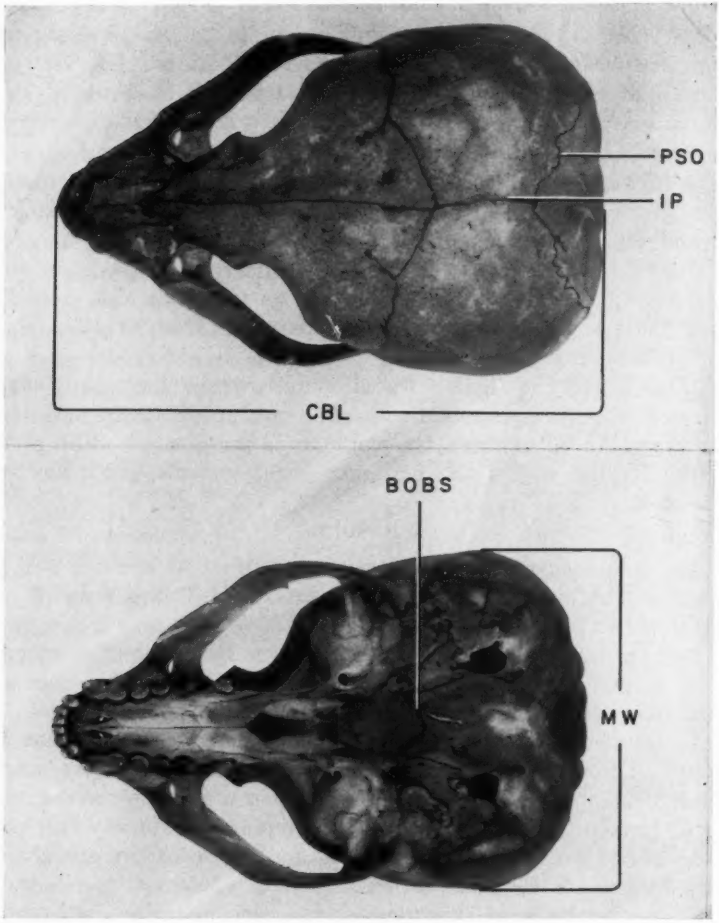

FIGURE 1

Skull of yearling female fur seal, collected 14 February at estimated age seven months. BOBS = basioccipital-basisphenoid suture, CBL = condylobasal length, IP = interparietal suture, MW = mastoid width, PSO = parietal-supraoccipital suture. (0.63 natural size).

TABLE 1
BODY WEIGHT OF FUR SEALS (KILOGRAMS)

Age in years	Number of specimens		Range		Mean		Standard deviation[1]		Coefficient of variation	
	Male	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*
0	23	16	4.1 - 7.1	3.3 - 6.0	5.4	4.8	0.9	0.7	16.7	14.6
¼	82	91	8.9 - 18.8	7.0 - 16.6	13.9	12.0	2.2	2.2	15.8	18.3
1-¼	12	6	12.5 - 22.0	12.9 - 15.2	16.8	14.1	2.4	1.0	14.3	7.1
2	5	8	17.3 - 24.3	14.5 - 22.2	20.9	16.3	3.4	2.5	16.3	15.3
3	90	9	21.8 - 35.4	16.8 - 25.0	28.0	21.2	3.1	2.9	11.1	13.7
4	26	7	27.2 - 48.1	19.5 - 27.2	35.5	23.2	5.2	4.0	14.6	17.2
5	28	3	30.9 - 57.2	26.3 - 30.2	44.9	28.5	7.2	2.0	16.0	7.0
6	22	4	46.3 - 83.5	20.9 - 37.2	60.7	29.9	10.3	6.6	17.0	22.1
7	6	2	69.0 - 156.6	31.8 - 36.8	94.5	34.3	32.2	3.5	34.1	10.2
8	4	3	80.8 - 222.5	24.5 - 47.2	175.5	36.0	65.3	11.3	37.2	31.4
9	1	2		39.0 - 41.8	188.4	40.4		2.0		4.9
10	4	5	163.4 - 269.2	34.5 - 40.9	202.6	38.9	46.4	2.7	22.9	6.9
>10	3	3	138.0 - 278.3	40.4 - 49.9	186.0	45.4	79.7	4.8	42.8	10.6
>10[2]		51		31.8 - 54.5		42.8		5.2		12.1
>10[3]		23		37.7 - 59.0		46.7		5.5		11.8

[1]SD calculated for all tables on basis of N-1.
[2]January specimens, 69% of them with fetus.
[3]March-April specimens, 74% of them with fetus.

on the threshold of sociological maturity rather than during, or slightly before, sexual maturity. That is, the weight is added at a period when the male is obliged to struggle with his rivals for a position on the breeding grounds. The male attains 81% of his weight after he has reached sexual maturity; the female only 50%. Generally speaking, the growth in weight of the female is slow and fairly uniform.

Weight is unreliable as a criterion of age because of overlapping. The largest suckling pups are heavier than the smallest yearlings; the largest yearlings are heavier than the smallest 3-year olds, and so on. In none of the five variates studied is there a distinct grouping of measurements according to age beyond the first summer of life.

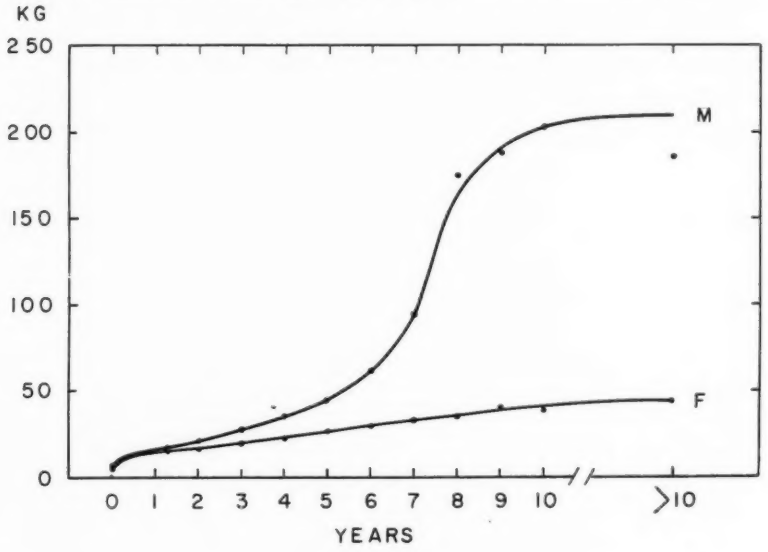

FIGURE 2
INCREASE IN BODY WEIGHT

Body length. Among mammals in general, length, because of its lesser variation, is a more reliable indication of size than is weight. On the basis of length, the newborn fur seal is relatively large. The male is one-third the length of the adult; the female one-half.

In the male, there seems to be a slight acceleration in body length in the seventh and eighth years corresponding to a growth in weight,

TABLE 2
BODY LENGTH OF FUR SEALS (MILLIMETERS)

Age in years	Number of specimens		Range		Mean		Standard deviation		Coefficient of variation	
	Male	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*
0	23	16	544 - 754	544 - 687	659	631	45.8	44.5	6.9	7.1
1-¼	12	6	890 - 1010	850 - 900	954	871	33.6	21.3	3.5	2.4
2	6	8	940 - 1121	898 - 1097	1044	977	63.7	36.0	6.1	3.7
3	90	9	1040 - 1250	1016 - 1170	1149	1081	47.9	57.2	4.2	5.3
4	26	7	1180 - 1388	1082 - 1203	1257	1122	54.5	38.9	4.3	3.5
5	28	3	1178 - 1472	1186 - 1245	1372	1207	66.1	32.9	4.8	2.7
6	22	4	1320 - 1586	1065 - 1250	1455	1148	62.3	28.2	4.3	2.5
7	6	2	1505 - 1851	1202 - 1213	1642	1207	147.3	7.9	9.0	0.7
8	4	2	1556 - 2019	1133 - 1255	1869	1194	212.8	86.3	11.4	7.2
9	1	1			1960	1266				
10	4	4	1850 - 2085	1168 - 1308	1941	1243	102.5	60.9	5.3	4.9
>10[1]	4	52	1870 - 2145	1177 - 1413	1985	1278	131.1	33.3	6.6	2.6
>10[2]		23		1205 - 1430		1312		57.9		4.4

[1]January specimens, 71% of them with fetus.
[2]March-April specimens, 74% of them with fetus.

although the data are inconclusive. This acceleration may, in fact, represent a sampling error. In the female, growth in length is slow and fairly uniform.

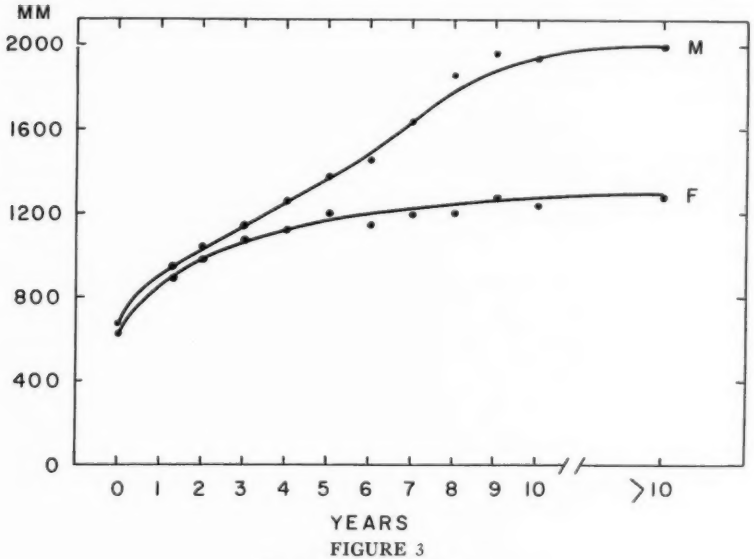

FIGURE 3
INCREASE IN BODY LENGTH

Hind flipper length. This measurement has a low coefficient of variation, 5 to 6%, comparable to that for body length, 4 to 5%, and lower than that for body weight, 14 to 17%. In both sexes the growth of the hind flipper is fairly uniform, somewhat slower in males than in females. It parallels rather closely the growth of body length, except that the hind flipper of the males does not show a spurt in growth during the seventh and eighth years.

Condylobasal length. This measurement is the least variable of the five, with a coefficient of variation of 2 to 4%. In males, the trend at age 13 suggests that the skull is still growing. In females, the skull is about as long at age 7 as it ever becomes.

Mastoid width. Variation is 3 to 6%. The data suggest even more strongly than do those for condylobasal length that growth of the

TABLE 3
HIND FLIPPER LENGTH OF FUR SEALS (MILLIMETERS)

Age in years	Number of specimens		Range		Mean		Standard deviation		Coefficient of variation	
	Male	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*
0	17	15	170 - 222	155 - 204	196	182	15.4	12.8	7.9	7.0
¾[1]		1				228				
1-¼	1				263					
2	6	8	255 - 306	261 - 305	290	285	26.1	17.0	9.0	6.0
3	237	9	300 - 399	287 - 356	341	317	18.8	19.2	5.5	6.1
4	26	7	335 - 423	320 - 377	374	346	21.2	21.3	5.7	6.2
5	28	3	372 - 460	352 - 388	424	365	24.8	20.0	5.8	5.5
6	21	4	381 - 465	320 - 374	431	335	25.5	26.0	5.9	7.8
7	6	2	451 - 492	382 - 387	474	385	18.4	5.1	3.9	1.3
8	4	2	448 - 539	367 - 411	397	389	39.4	31.1	7.9	8.0
9	1	1			494	426				
10	4	3	485 - 540	377 - 396	517	388	26.6	9.8	5.1	2.5
>10[2]	3	24	525 - 567	352 - 434	548	397	21.1	22.5	3.9	5.7

[1]Collected 11 April.
[2]March-April specimens from Japanese waters.

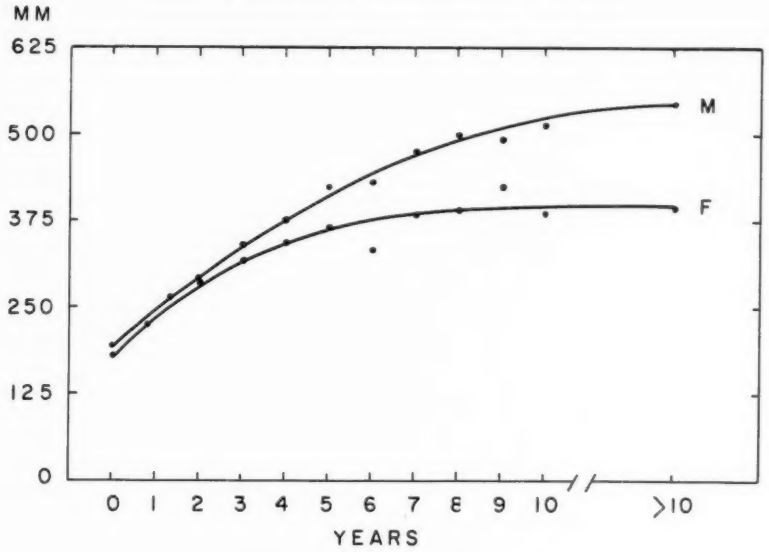

FIGURE 4
INCREASE IN HIND FLIPPER LENGTH

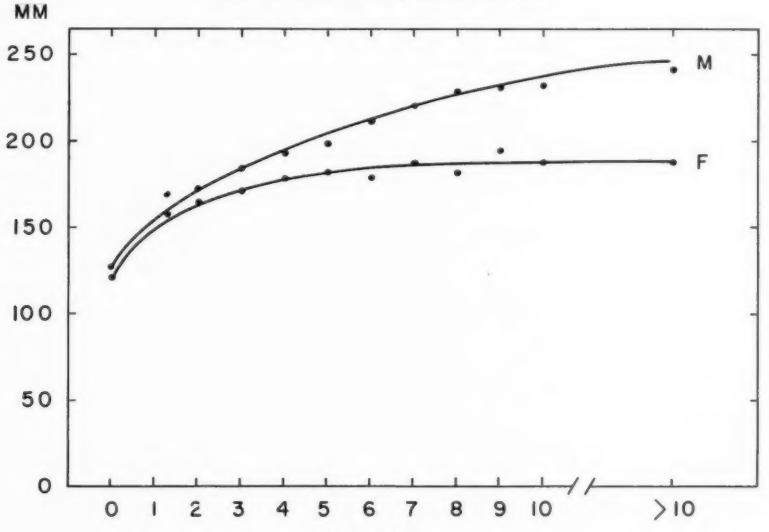

FIGURE 5
INCREASE IN CONDYLOBASAL LENGTH

TABLE 4
CONDYLOBASAL LENGTH OF FUR SEALS (MILLIMETERS)

Age in years	Number of specimens		Range		Mean		Standard deviation		Coefficient of variation	
	Male	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*
0	8	7	120 - 130	117 - 123	126	120	3.5	2.0	2.8	1.7
1-¼	11	6	158 - 179	152 - 162	169	158	6.5	3.6	3.8	2.3
2	11	7	166 - 176	158 - 173	172	164	3.6	3.4	2.1	2.1
3[1]	38	8	177 - 192	163 - 179	184	170	4.3	5.4	2.3	3.2
4[1]	21	8	179 - 206	168 - 197	192	178	7.4	8.4	3.9	4.7
5	13	3	190 - 203	179 - 186	199	182	3.7	3.0	1.9	1.6
6	19	4	199 - 221	172 - 188	211	179	6.0	7.2	2.8	4.0
7	6	2	212 - 231	187 - 188	220	187	7.5	1.4	3.4	0.7
8	3	2	218 - 235	176 - 185	229	181	9.8	6.4	4.3	3.5
9	1				232					
10	1	1			228	194				
10[3]		8		181 - 194		188		4.0		2.1
adult[3]	67		213 - 262		241		8.9		3.7	
>10		41		178 - 199		189		4.5		2.4

[1]Screened by clubbers on basis of body length.
[2]Age estimated from tooth-ridge counts.
[3]Recorded in the field as "adult" or "bull"; estimated ages 7 to 30 years.

male skull continues far past middle life. In females, the skull ceases to grow in width at age seven or eight.

Measurements made by Rand (1950) on skulls of the southern fur seal, *Arctocephalus,* indicate that growth in condylobasal length and mastoid width of the oldest individuals had ceased. The ages of Rand's specimens were estimated, not chronologically but physiologically, in terms of suture closure.

The newborn northern fur seal skull is at least 51% as long, and up to 85% as wide, as it will ultimately become.

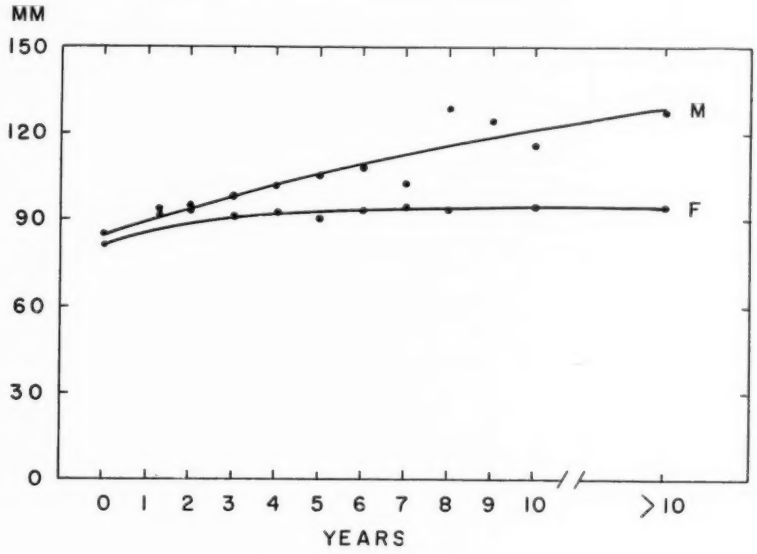

FIGURE 6
INCREASE IN MASTOID WIDTH

Skull sutures. As a possible clue to age, the rate of suture closure in the seal skull was briefly scrutinized. Three prominent sutures were selected (Figure 1 and Table 7). All are open at time of birth and all are closed before age 7. The basioccipital-basisphenoid suture closes in the male between the second and sixth year; in the female rather abruptly in the second or third year. The parietal-supraoccipital closes in both male and female seals between the second and sixth

TABLE 5
MASTOID WIDTH OF FUR SEALS (MILLIMETERS)

Age in years	Number of specimens		Range		Mean		Standard deviation		Coefficient of variation	
	Male	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*
0	8	6	81 - 89	78 - 84	85	81	2.3	2.5	2.7	3.1
1-¼	11	6	90 - 99	87 - 95	93	91	2.5	3.3	2.7	3.6
2	11	7	90 - 99	89 - 98	94	93	2.7	3.2	2.9	3.4
3[1]	39	8	88 - 112	86 - 94	98	91	5.3	2.6	5.4	2.9
4[1]	21	7	91 - 112	89 - 99	101	92	6.6	3.2	6.5	3.5
5	12	3	96 - 113	87 - 93	105	90	5.1	3.1	4.9	3.4
6	20	4	96 - 119	90 - 95	109	93	5.3	2.2	4.9	2.4
7	6	2	104 - 125	94 - 95	111	95	7.8	1.4	7.0	1.5
8	4	2	104 - 141	90 - 95	129	93	16.7	3.6	12.9	4.0
9	1				125					
10	1	1			116	98				
10[2]		8		89 - 97		94		3.0		3.2
adult[3]	67		110 - 146		128		7.2		5.6	
>10		41		90 - 102		95		3.1		3.3

[1]Screened by clubbers on basis of body length.
[2]Age estimated from tooth-ridge counts.
[3]Recorded in the field as "adult" or "bull"; estimated ages 7 to 30 years.

TABLE 6

RELATIVE GROWTH IN THE FUR SEAL

(Size at birth and at sexual maturity expressed as percentage of full-grown size)

		Newborn		Sexually mature[1]		Full-grown[2]	
		Male	*Female*	*Male*	*Female*	*Male*	*Female*
Body weight	kg	5.4	4.8	32	22	210	44
	% full-grown size	3	11	19	50	100	100
Body length	mm	659	631	1203	1101	2000	1295
	% full-grown size	33	49	66	85	100	100
Hind flipper length	mm	196	182	357	331	548	400
	% full-grown size	36	45	73	83	100	100
Condylobasal length	mm	126	120	188	174	245[3]	189
	% full-grown size	51	63	80	92	100	100
Mastoid width	mm	85	81	100	91	130[3]	95
	% full-grown size	65	85	79	96	100	100

[1]Sexual maturity is attained at age 4 to 5 in most males, 3 to 4 in most females. The mean measurements for these ages are used above.

[2]The adult size is considered to be the asymptote of the growth curve for each variate except male skull size.

[3]The asymptote for male skull size is uncertain; perhaps higher than these values; see discussion in text.

year. The interparietal closes in the male between the fourth and seventh year; in the female between the third and seventh year. In *Callorhinus,* then, suture closure is an indication of age within wide limits only and is no more reliable for this purpose than body length or skull length.

Sex discrepancy in size. (Table 8 and Figure 7) In length of body and hind flipper, and length and width of skull, the male fur seal is 4 to 8% larger than the female when born and about 40% larger when full-grown. The male is 12% heavier than the female when born and 370% heavier when full-grown. That is, on an average, the full-grown bull is about four-and-one-half times as heavy as the full-grown cow.

Comparisons with other mammals. This sex discrepancy is possibly the greatest of its kind among mammals. It is approached by the northern sea-lion (*Eumetopias*) in which the male is nearly four

TABLE 7
CLOSING OF SKULL SUTURES ACCORDING TO AGE
(PERCENTAGE OF SKULLS HAVING CLOSED SUTURES)

Age in years		Basioccipital-basisphenoid suture		Parietal-supraoccipital suture		Interparietal suture	
		Male	*Female*	*Male*	*Female*	*Male*	*Female*
1	N	11	6	11	6	11	6
	%	*0*	*0*	*0*	*0*	*0*	*0*
2	N	10	7	10	4	10	7
	%	*40*	*71*	*10*	*25*	*0*	*0*
3	N	39	8	39	8	39	8
	%	*64*	*100*	*72*	*87*	*0*	*37*
4	N	21	8	20	8	22	8
	%	*76*	*100*	*65*	*87*	*5*	*37*
5	N	13	3	13	3	13	3
	%	*85*	*100*	*92*	*100*	*23*	*100*
6	N	21	4	21	4	21	4
	%	*100*	*100*	*100*	*100*	*57*	*75*
7	N	6	2	6	2	6	2
	%	*100*	*100*	*100*	*100*	*100*	*100*

TABLE 8
SEX DISCREPANCY IN SIZE[1]

	Body weight, kg	Body length, mm	Hind flipper length, mm	Condylo-basal length, mm	Mastoid width, mm
Male, newborn	5.4	659	196	126	85
Male, full-grown	210	2000	548	245	130
Female, newborn	4.8	631	182	120	81
Female, full-grown	44	1295	400	189	95
Ratio, newborn male/female	1.1	1.0	1.1	1.0	1.0
Ratio, full-grown male/female	4.7	1.5	1.4	1.3	1.4

[1]For each variate, the size of the newborn is the mean of observed measurements; the size of the adult is the asymptote by extrapolation from the growth curve.

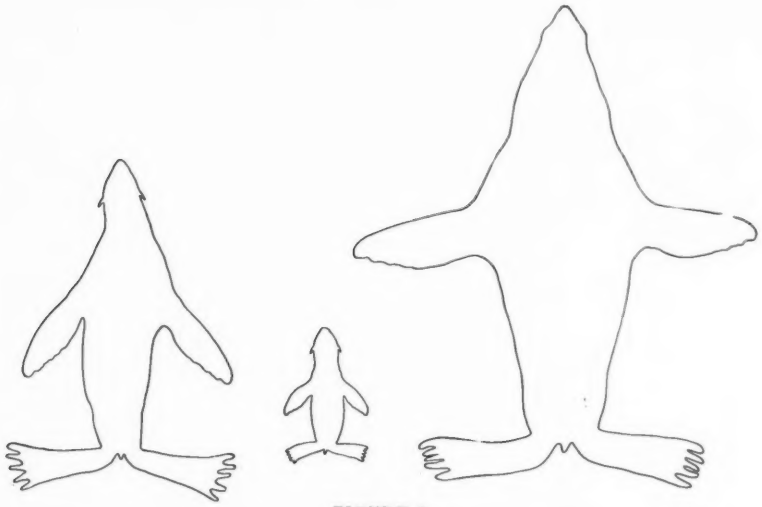

FIGURE 7

Relative size of newborn and full-grown fur seals; body outlines drawn to the same scale from photographs. Left to right:
Full-grown female, age 10, length 1272 mm, weight 40 kg.
Newborn female, length 544 mm, weight 3.3 kg.
Full-grown male, age 8, length 1915 mm, weight 184 kg.

times the weight of the female (e.g., male 2,096 lbs., female 565 lbs.) and the southern fur seal in which the male is three times the weight of the female (average males 503 lbs., females 170 lbs., according to Rand, 1950: pt. 1, tbl. 2; pt. 3, tbl. 3). The female elephant seal, *Mirounga leonina,* is said to be "only about one quarter of the bulk" of the male (Sorensen, 1950: 7). There appear to be no actual recorded weights of full-grown elephant seals, largest of all pinnipeds. A reasonable estimate of the maximum weight of the male would be 4 to 5 metric tons, based on length/weight relationships of subadults and lengths of adults.

Summary

The maximum life span of the fur seal, *Callorhinus ursinus,* is believed to be 30 years. Newborn males average in weight 5.4 kg. and females 4.8 kg. Both sexes grow steadily until year 7 or 8, when the female attains full size and the male begins suddenly to grow. When

full-grown, year 13 or more, the males average 186 kg., or 4-1/2 times the weight of the females. Newborn males average in body length 659 mm. and females 631 mm. When full-grown, males average 1,985 mm. and females 1,278 mm. The growth of the hind flipper parallels that of body length. Dentinal layers on the teeth show that growth is slower at one season of the year (probably in summer). The skull of the male apparently continues to grow after mid-life. The rate of suture closure is variable and, like body size, is a poor indication of age.

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PARTIAL PURIFICATION OF GROWTH FACTORS IN THE DIALYZABLE FRACTION OF CHICK EMBRYO EXTRACT[1]

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INTRODUCTION

The growth-promoting action of embryonic extract for animal cells in tissue culture is well-known from the early work of Carrel and his associates beginning in 1913, and from the numerous studies made subsequently by others (see Fischer 1946, Parker, 1950). Much of this work has been concerned with protein factors and other substances of high molecular weight. Carrel stressed the lability of factors associated with the protein fraction and was inclined to attribute the effect of embryo extract to proteoses or other protein cleavage products (Carrel and Baker, 1926). Fischer made numerous investigations of proteins from chick embryos (Fischer, 1930, 1946; Fischer and Astrup, 1943) and eventually isolated an active nucleoprotein fraction using the method of Hammersten (1920). This result was later confirmed by Davidson and Waymouth (1945). Recently Nishioka and Ibuka (1952) have described a procedure for the isolation of high-polymer pentose nucleic acid from liver. Katsuta and Takaoka (1952) report the preparations obtained by this method from chick embryos promote growth in tissue culture. However Kutsky (1953) in this laboratory was unable to confirm the findings of the Japanese workers although considerable growth stimulation was consistently obtained with a nucleoprotein fraction. The latter was prepared by a novel procedure involving the use of streptomycin as a selective precipitating agent.

Other investigations have focused on the growth-promoting effect of fractions from embryo extract containing only factors of low molecular weight. The stimulating action of dialysates (Fischer 1941, 1946; Harris 1952a, 1952b) and ultrafiltrates (Stewart, 1950; San-

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ford, Waltz, Shannon, Earle and Evans, 1952; Rosenberg and Kirk, 1953, Rosenberg 1953) has been clearly established in a variety of protein-containing assay systems, although no activity has been demonstrated in the absence of protein. Indeed, Fischer (1946) has maintained that the action of embryo extract is a composite effect involving factors of high molecular weight ("embryonin") as well as dialyzable materials ("accessory growth factors"). Evidence supporting this concept has been presented by Wright (1926), Margoliash, Tenenbaum, and Doljanski (1948) and Harris (1952a), and recently we have been able to demonstrate directly a synergism between dialysate and nucleoprotein fractions isolated from chick embryos by appropriate procedures (Harris and Kutsky, 1953). On the other hand Sanford et al. (1952) found that a combination of embryo extract ultrafiltrate and serum residue produced increases in cell number as great as those obtained with unfractionated embryo extract alone, and on this basis have questioned the obligatory role of nucleoprotein factors.

The nature of the active growth factors in dialysates or ultrafiltrates from embryo extract is still obscure. Fischer and associates attributed this action to free amino acids, especially cystine, and to an additional small group of nutrients of which glutamine, glycine, fructose diphosphate, and hypoxanthine, have been most recently emphasized (Fischer 1941, 1946, 1948; Ehrensvärd, Fischer and Stjernholm, 1949). The experimental basis for these conclusions however, has been criticized by White and Lasfargues (1949) and Harris (1952a) on technical grounds. Using media dialyzed under conditions of stabilized pH and buffer capacity Harris (1952b) found no stimulating effect from cystine or amino acid supplements when tested alone, and a rise in amino nitrogen in unsupplemented media on incubation. Likewise a series of co-factors including fructose diphosphate as well as a variety of nucleic acid derivatives were inert in this assay system, and did not duplicate the clear-cut stimulation obtained by supplementing the dialyzed assay medium with dialysates of chick embryo extract.

It seems apparent that direct fractionation of dialysates or ultrafiltrates offers the most promising means for establishing the nature of the active factors contained in these materials. In a preceding paper (Harris 1952b) we have described a crude extract prepared from chick embryos by whole extraction and dialysis. The crude

dialysate in assay cultures produces large increases in surface area accompanied by characteristic changes in cell morphology and a rise in glycolytic activity. It is the purpose of the present communication to describe a partial purification of the crude extract by precipitation with barium and ethanol, using modifications of a method outlined by Umbreit, Burris and Stauffer (1945) for the analysis of phosphorylated intermediates.

Preparation of Fractions

The starting material for fractionation procedures to be described was obtained by homogenizing 12-day chick embryos in ethanol to a final concentration of 70%, extraction of the supernatant with chloroform, dialysis of the aqueous fraction, and evaporation of the pooled dialysates over $CaCl_2$. Details are given in a preceding paper (Harris, 1952b). The crude dialysate was reconstituted in a small volume of double-distilled water (15.0 ml./gm. dry dialysate) and adjusted to pH 8.2 with 0.1N-NaOH using a Beckman pH meter, Model G. The solution was then treated with 1M-$BaCl_2$ also adjusted to pH 8.2 (2.5 ml./gm. dry dialysate) and the resulting preparation chilled at O°C. for 30 minutes. After centrifugation at 3000 RPM for 10 minutes in an International Centrifuge, Type SB, the supernatant was decanted and the precipitate washed three times. The residual pellet was set aside as the "*barium-insoluble fraction.*" The original supernatant ("*barium-soluble fraction*") was then chilled to O°C. and further subdivided by adding four volumes of cold absolute ethanol (final concentration 80%). After 30 minutes in an ice bath the resulting precipitate ("*alcohol-insoluble fraction*") was centrifuged off, the supernatant ("*alcohol-soluble fraction*") decanted and the precipitate washed three times with cold 80% ethanol. It is essential to maintain the precipitate at low temperatures throughout the alcohol separation in order to avoid alterations in physical consistency and presumably chemical composition of the alcohol-insoluble material. The final pellet was rinsed into an evaporating dish with a small amount of double distilled water and was evaporated to dryness over $CaCl_2$ with very gentle heat from an infra-red lamp. The alcohol-soluble supernatant fraction was similarly evaporated to dryness and subsequently was extracted with a small volume of 80% ethanol (10 ml./gm. original dry dialysate), centrifuged to leave behind a pellet of excess salt, and the supernatant again evaporated

to dryness. The three fractions obtained by the foregoing procedure (barium-insoluble, alcohol-insoluble, alcohol-soluble) were finally reconstituted separately in small volumes of 0.05N-HCl, Ba^{++} removed by treating with a sight excess of 1M-Na_2SO_4 and centrifuging, and the solutions neutralized with 0.1N-NaOH. After appropriate dilution with glucose-free Gey's solution each fraction was sterilized for assay purposes by passage through a sintered glass filter (ultrafine).

Assays in Tissue Culture

Culture methods. All cultures were made in Carrel D-3.5 flasks using fresh explants obtained from 12-15 day chick heart. The medium consisted of a chicken plasma clot with supernatant made up from horse serum, chick embryo extract, and Gey's saline in varying proportion as outlined below. Each of these components was subjected to dialysis against a balanced saline (F-2 solution) before use, to reduce the concentration of dialyzable growth factors to a minimum in the final assay medium. The cultures were incubated at 38°C. for 10 days with biweekly change of fluid phase, without washing. Details of culture methods, technique of dialysis and the generalized use of dialyzed media for studies in cell nutrition are covered in preceding papers (Harris 1952a, 1952b).

Activity of fractions in plasma-serum assay medium. The initial assays were carried out in a medium containing minimal levels of embryo extract. Explants were placed in dialyzed plasma (0.5 ml./flask) and coagulation induced by adding 1.0 ml. Gey's solution containing 0.02 ml. dialyzed embryo extract (non-dialyzable components) to each flask. Later 1.0 ml. supernatant was added (40% dialyzed horse serum, 60% Gey's solution) to complete the medium. Test fractions were incorporated at appropriate levels in the Gey's solution. Relative concentrations of test fractions were expressed in terms of a corresponding amount of initial unfractionated dialysate, since a variable salt content after removing Ba^{++} made direct weights of the fractions unreliable. The effects of each fraction were studied individually as well as in combination in the assay medium.

Table 1 presents a plan for this group of experiments. Gross observations and terminal measurements of surface area indicated that the dialysate factors active in promoting outgrowth were barium-soluble and were distributed between alcohol-soluble and alcohol-insoluble

TABLE 1

INFLUENCE OF SUBFRACTIONS FROM CRUDE ALCOHOLIC EXTRACT ON OUTGROWTH OF CHICK HEART FIBROBLASTS

I. Effects in dilute serum assay medium*

Fractions added		Experiment I		Experiment II		Experiment III	
		Relative concentration†	Areal increase‡ at 10 days, mm^2	Rel. conc.†	Areal increase‡ at 10 days, mm^2	Rel. conc.†	Areal increase‡ at 10 days, mm^2
None (control)		—	32.1 ± 7.7	—	18.8 ± 2.3	—	20.8 ± 6.9
Barium-insoluble (A)		—	—	2X	21.8 ± 4.3	2X	11.9 ± 1.3
Barium-soluble	Alcohol-insoluble (B)	1X	24.4 ± 1.9	1X	41.5 ± 7.8	1X	40.1 ± 12.8
		2X	26.1 ± 2.1	2X	50.4 ± 4.1	2X	49.8 ± 7.9
	Alcohol-soluble (C)	1X	48.8 ± 3.6	1X	32.7 ± 5.5	1X	34.5 ± 9.9
		2X	45.3 ± 3.8	2X	50.1 ± 10.2	2X	29.2 ± 5.3
(B) + (C)		1X	90.1 ± 17.7	1X	100.2 ± 12.5	1X	126.6 ± 12.5
		2X	84.8 ± 7.0	2X	75.6 ± 12.9	2X	109.9 ± 11.9
(A) + (B) + (C)		—	—	2X	73.9 ± 8.1	2X	110.5 ± 14.8
Crude extract (unfractionated)		1X	86.0 ± 4.9	1X	89.5 ± 15.1	1X	136.4 ± 19.2

* *Total medium:* dialyzed chicken plasma clot + supernatant 40% dialyzed horse serum and 60% Gey's solution.

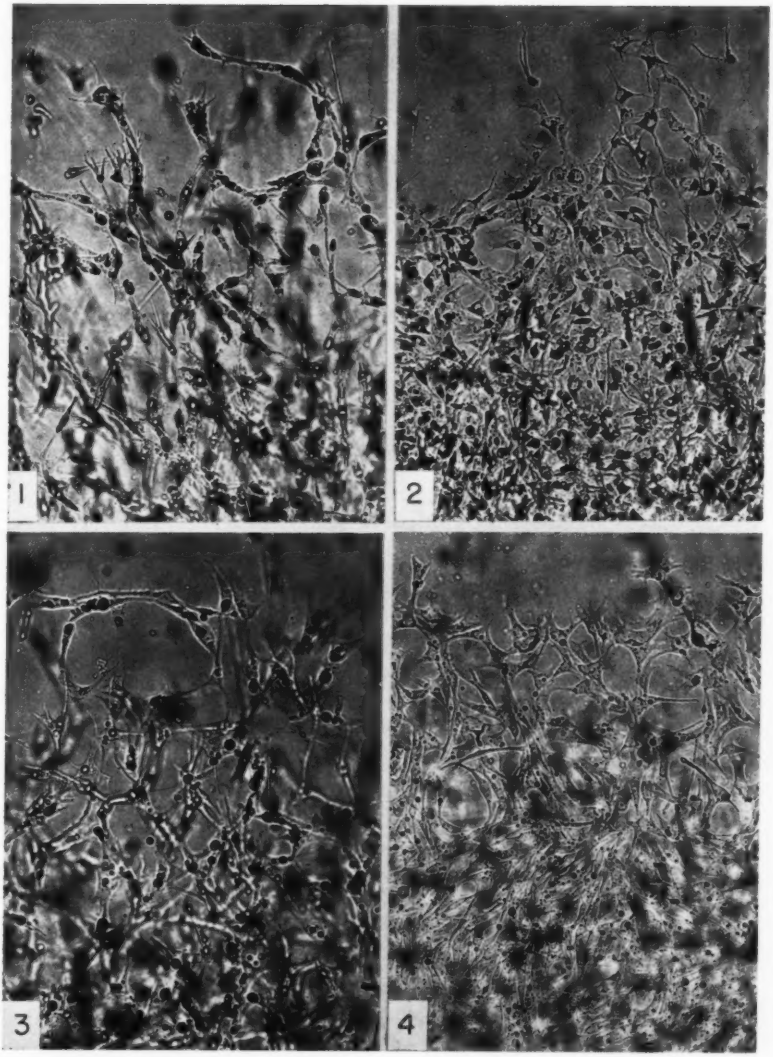
† In terms of amount of crude extract from which each fraction was derived. For crude extract, 1X = 2.2 mg./ml. assay medium. See Table 4 for analyses of crude extract and active subfractions.

‡ Mean value and standard error (Snedecor, 1946). Six cultures in each group.

fractions. Neither of these two fractions produced large outgrowths when assayed singly but in combination the increase in surface area was as great as that obtained with unfractionated crude dialysate. Variation in concentration levels (Table 1) indicated that the interaction of the two fractions was not based on a simple additive effect from a common factor. On the other hand the barium-insoluble fraction was inert singly and did not augment the overall effect when combined with the other two components of the original extract. The barium-insoluble fraction for tissue extracts in general, if prepared at low temperatures, contains adenosine triphosphate, hexose diphosphate, and inorganic phosphate (Umbreit, et al., 1945). These factors have previously been reported not limiting for the reduction in outgrowth produced by dialysis (Harris, 1952b). The dispensability of the barium-insoluble fraction in the present experiments is in line with this observation and suggests that the active dialysate factors do not include ATP, HDP, or inorganic phosphate.

Microscopic study of cell morphology and growth patterns disclosed differences which paralleled in general the effects of test fractions on surface area (Plate I, Figures 1-4). Outgrowths in unsupplemented basal medium were restricted to a narrow rim of jagged, refractile cells and these were widely spaced with large central vacuoles. Substantially identical outgrowths were observed in cultures containing barium-insoluble supplements alone. The alcohol-insoluble fraction when added singly produced a dense population of cells which became refractile and filled with inclusions after a few days. Outgrowths in cultures containing the alcohol-soluble fraction alone often spread more widely than the unsupplemented controls but exhibited a similar appearance with open networks of skeletonized, refractile cells. Optimal results were obtained in cultures with both alcohol-soluble and insoluble components present. These outgrowths consisted of broad flat cells, not refractile and similar to outgrowths obtained with unfractionated crude dialysate.

Another index of stimulation by test fractions was provided in some cases by measurements of glycolytic activity in assay cultures. Lactic acid determinations were made on supernatant fluids from 8-day cultures by the method of Barker and Summerson (1941), using the Beckman spectrophotometer. These results as shown in Table 2 further document the stimulating effect of dialysate factors and



FIGURES 1-4. Cellular detail at 5 days in cultures of chick heart fibroblasts. Dialyzed plasma-serum saline medium. X 81

FIGURE 1. Unsupplemented basal medium.

FIGURE 2. Basal + alcohol-insoluble fraction.

FIGURE 3. Basal + alcohol-soluble fraction.

FIGURE 4. Basal + alcohol-insoluble and alcohol-soluble fractions.

All photomicrographs taken with the assistance of Mr. Victor Duran.

TABLE 2
LACTIC ACID IN SUPERNATANT FLUIDS OF 8-DAY CULTURES OF CHICK HEART FIBROBLASTS*

Fractions added	Relative concentration†	Lactic acid micrograms/ml.**
None (control)	—	271
		265
Unfractionated crude dialysate	1X	435
		423
Alcohol-insoluble fraction	1X	370
		368
Alcohol-soluble fraction	1X	367
		350
Alcohol-insoluble +		586
alcohol-soluble fractions	1X	576

* *Basal assay medium:* dialyzed chicken plasma clot + supernatant 40% dialyzed horse serum and 60% Gey's solution.
**Duplicate determinations on pooled supernatants from six cultures in each series.
† See footnote with Table 1.

show again the interaction of alcohol-soluble and alcohol-insoluble fractions.

Activity of fractions in media containing dialyzed embryo extract. Experiments were also performed to study the activity of test fractions in the presence of the non-dialyzable components in chick embryo extract. For this purpose 15% dialyzed chick embryo extract was incorporated in the basal medium, which also contained 40% dialyzed horse serum in the fluid phase and a dialyzed plasma clot as before. Table 3 summarizes the studies carried out in this group of experiments. Outgrowths were minimal in unsupplemented basal medium although markedly larger than those obtained in the absence of embryo extract (see Table 1). Addition of the crude unfractionated dialysate produced large increases in surface area and a similar effect could be obtained by supplementing with alcohol-soluble plus alcohol-insoluble fractions. On the other hand, outgrowths nearly as large were produced by adding the alcohol-soluble fraction alone, while the alcohol-insoluble fraction when tested singly was actually inhibiting. The barium-insoluble fraction had no effect on outgrowth whether added to the assay medium alone or in combination.

The above effects on surface area were correlated with changes observed at the microscopic level. In control cultures (unsupplemented basal medium) the cells became enlarged, refractile, and filled with vacuoles (Harris, 1952a). These changes were accelerated and accentuated by adding the alcohol-insoluble fraction alone. In the pres-

TABLE 3

INFLUENCE OF SUBFRACTIONS FROM CRUDE ALCOHOLIC EXTRACT ON OUTGROWTH OF CHICK HEART FIBROBLASTS

II. Effects in assay medium containing 15% dialyzed embryo extract (non-dialyzable components)*

		Experiment I		Experiment II		Experiment III	
		Relative concentration†	Areal increase‡ at 10 days, mm^2	Rel. conc.†	Areal increase‡ at 10 days, mm^2	Rel. conc.†	Areal increase‡ at 10 days, mm^2
None (control)		—	54.8 ± 3.0	—	79.8 ± 7.3	—	52.6 ± 4.8
Barium-insoluble (A)		2X	48.6 ± 4.3	2X	63.9 ± 3.8	—	—
Barium-soluble	Alcohol-insoluble (B)	1X	44.7 ± 1.9	1X	56.7 ± 3.1	1X	50.8 ± 1.5
		2X	47.4 ± 0.5	2X	54.8 ± 4.2	2X	46.6 ± 2.3
	Alcohol-soluble (C)	1X	92.9 ± 2.6	1X	—	1X	83.3 ± 4.6
		2X	89.5 ± 7.0	2X	127.1 ± 9.3	2X	—
(B) + (C)		1X	98.1 ± 6.4	1X	126.8 ± 6.0	1X	94.2 ± 5.3
		2X	—	2X	141.9 ± 11.3	2X	111.5 ± 4.3
(A) + (B) + (C)		1X	92.4 ± 5.6	1X	150.3 ± 12.1	1X	92.0 ± 5.4
Crude extract (unfractionated)		1X	—	1X	—	1X	117.0 ± 10.0
		2X	—	2X	130.3 ± 10.5	2X	—

* *Total medium:* dialyzed chicken plasma clot + supernatant 40% dialyzed horse serum, 15% dialyzed embryo extract (EE50), 45% Gey's solution.

† In terms of amount of crude extract from which fraction was derived. For crude extract, 1X = 2.2 mg./ml. assay medium. See Table 4 for analyses of crude extract and active subfractions.

‡ Mean value and standard error (Snedecor, 1946). Six cultures in each group.

ence of either crude dialysate, alcohol-soluble + alcohol-insoluble fractions, or alcohol-soluble fraction alone a dense, broad outgrowth zone was observed. In these cultures the cells remained small, without conspicuous inclusions and with the delicate, non-refractile processes characteristically seen in undialyzed media. No significant changes at the microscopic level were associated with addition of the barium-insoluble fraction.

The data obtained thus suggest that in the presence of the non-dialyzable fraction of embryo extract optimal outgrowths are obtained by the addition of a single fraction (alcohol-soluble) of the crude dialysate. This result contrasts sharply with the apparent requirement of both alcohol-insoluble fractions for optimal outgrowth in the absence of dialyzed embryo extract (Table 1). It is apparent that the specific proteins or other non-dialyzable components of the medium may determine in part the requirement for dialyzable factors.

In addition it should be emphasized that the density and apparent thickness of outgrowths in media containing dialyzed embryo extract were appreciably greater than those obtained in its absence. Although cultures in a plasma-serum medium if supplanted by crude dialysate often reached a considerable size (Table 1) the outgrowth zones were characteristically thin, nearly transparent. This observation is consistent with the interaction of dialyzable and non-dialyzable factors in embryo extract as postulated by Fischer (1946) and others.

Characterization of Fractions

Analytical data.—Determinations of total nitrogen and phosphorus in the active dialysate fractions are shown in Table 4. Nitrogen determinations were performed by Mr. B. W. Grunbaum using a

TABLE 4
Nitrogen and Phosphorus Content of Active Dialysate Fractions

Fraction	Total N,* micrograms per mg. crude dialysate	Total P,* micrograms per mg. crude dialysate
Unfractionated dialysate	50	13.
	48	14.
Alcohol-insoluble fraction	13	8.2
	11	8.5
Alcohol-soluble fraction	24	1.6
	24	1.7

* Duplicate determinations.

modified Kjeldahl procedure (Grunbaum, Schaffer, and Kirk, 1952). Dr. F. L. Schaffer carried out phosphorus measurements utilizing a sealed tube digestion method followed by spectrophotometric determination (Schaffer, Fong, and Kirk, 1952). The combined N and P values for alcohol-soluble and alcohol-insoluble fractions were lower than those of the crude extract, since inorganic phosphate and other barium-insoluble materials were discarded. No data were obtained on the barium-insoluble fraction inasmuch as this component was inert in culture. The alcohol-insoluble fraction contains most of the remaining organic phosphorus, as for tissue extracts in general (Umbreit et al., 1945). The higher level of nitrogen in the alcohol-soluble fraction reflects a high concentration of free amino acids (see below).

Ultraviolet absorption spectra.—Absorption curves were obtained for the crude extract and two active fractions using a Beckman spectrophotometer. Solutions were prepared in distilled water and contained the equivalent of 2 mg./ml. crude extract in each case. Figure 5 shows the characteristic curves obtained with the two active fractions. The alcohol-insoluble component is distinguished by a prominent peak at 260 mμ indicating the presence of purine or pyrimidine compounds. The selective absorption in this region is much less for the alcohol-soluble fraction. Although no curve is shown for the unfractionated dialysate, the pattern obtained was intermediate between that of the two active fractions.

Preliminary chromatographic separations.—For purposes of chromatography the excess salt was removed from active dialysate fractions by solvent extraction. Aliquots of alcohol-soluble and alcohol-insoluble fractions after drying were treated with acetic-methanol (2 vols. absolute methanol : 1 volume glacial acetic acid) and centrifuged. The pellets containing excess salt were discarded, the supernatants diluted 50% with distilled water and extracted in separatory funnels with absolute ether to remove methanol and acetic acid. The final water phases were evaporated to dryness over $CaCl_2$. Through the courtesy of Dr. Phyllis B. Kutsky the extracted fractions were then chromatographed on eight inch squares of filter paper, using a capillary-ascent method (Williams and Kirby, 1948). The solvents used were phenol saturated with water and a mixture of n-butyl alcohol, acetic acid and water (approximately 8:2:3). Although it was not possible to make a complete identification of ninhydrin-positive compounds in these chromatograms, the results in-

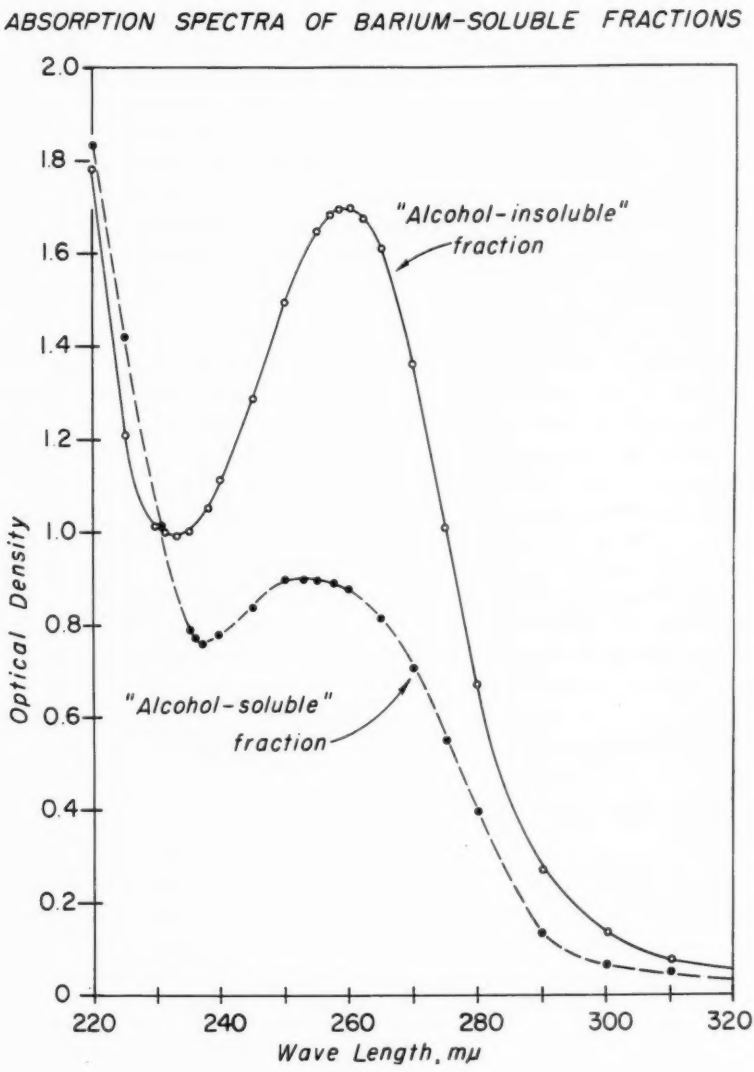

ABSORPTION SPECTRA OF BARIUM-SOLUBLE FRACTIONS
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Optical Density
"Alcohol-insoluble" fraction
"Alcohol-soluble" fraction
220
240
260
280
300
320
Wave Length, mμ

FIGURE 5

dicated significant differences between the two fractions respectively. Glycine, glutamic acid, glutamine, serine, and aspartic acid were found in both. Tyrosine, valine-methionine, leucine-isoleucine, phenylalanine, proline, and arginine were restricted to the alcohol-soluble fraction while alanine and threonine occurred principally in this material. A characteristic spot found only in the alcohol-insoluble fraction proved on elution and hydrolysis to be glutathione. No sulfhydryl compounds could be demonstrated in the alcohol-soluble fraction.

Assays with glutathione and cystine.—In accordance with the data obtained by chromatography, cultures were established in a plasma-serum assay medium to explore the effects of glutathione in the present assay system. The results obtained suggested that glutathione or a related compound may account for a significant part of the activity previously demonstrated in the alcohol-insoluble fraction. Thus when added singly at 20γ/ml. glutathione did not produce increases in surface area, while the cells became increasingly refractile and filled with small vacuoles, as in cultures supplemented with alcohol-insoluble fraction alone (see p. 152). Addition of glutathione with the alcohol-insoluble fraction did not significantly affect the picture obtained with the latter singly. On the other hand when glutathione at 20γ/ml. was combined with the alcohol-soluble fraction the resulting cultures showed prominent outgrowth zones, increased production of lactic acid, and the characteristic improvements in cell morphology previously found only in cultures supplemented with crude dialysate or alcohol-soluble + insoluble fractions. An active role for glutathione in embryo extract is consistent with the observation of Rapkine and Ephrussi (Ephrussi, 1932) that embryo extract is rich in —SH groups, and according to Borger and Peters (1933) the amount of reduced glutathione in fresh extracts is as high as 30 mg. per 100 ml.

We have also found, however, that cystine at a level of 15γ/ml. elicits effects parallel to those described for glutathione. When tested alone cystine is consistently inhibiting in our assay systems (Harris, 1952b) but recent experiments indicate clearly a synergism with the alcohol-soluble fraction similar to that obtained with glutathione. With neither cystine or glutathione plus alcohol-soluble fraction is the increase in surface area as great as with crude dialysate alone nor are the changes in cell morphology as clear-cut. It is not yet clear whether this difference is merely one of proper concentrations and proportions,

or whether additional essential agents remain to be characterized in the alcohol-insoluble material. In any event the use of ion exchange methods (Rosenberg and Kirk, 1953) offers the best promise for further definition of growth factors in the two dialysate fractions.

Discussion

The present experiments indicate that the active growth factors in crude dialysates of chick embryo-extract are barium-soluble in aqueous solution and can be divided into two groups by differential solubility of the barium salts in 80% ethanol. The only similar published studies of an analytical character are those of Fischer, Astrup, Ehrensvärd, and Oehlenschläger (1948). These investigators, working with dialysates obtained from calf embryos minced in Ringer's solution, reported that all of the active substances could be precipitated from solution with barium acetate. This result, however, was obtained only in the presence of 50% ethanol, and is thus not necessarily in conflict with our finding that the active factors are barium-soluble in aqueous solution. No experiments were performed by Fischer et al. to determine differential solubility of the barium salts in ethanol.

It is apparent that the assay system is of critical importance for evaluating the effect of dialysate factors on cells *in vitro*. In the present experiments both alcohol-soluble and insoluble fractions were required to elicit an optimal stimulation if the dialyzed basal medium did not contain embryo extract. With the addition of the non-dialyzable component of embryo extract, however, the alcohol-insoluble fraction became dispensable. This difference emphasizes the caution which must be used in characterizing basic requirements until more information is available concerning the interaction of protein and non-protein growth factors in the assay medium.

Acknowledgments

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Summary

1. Extracts were prepared by homogenizing 12-day chick embryos in ethanol followed by dialysis and evaporation of the dialysate fraction.

2. The crude dialysate in aqueous solution was fractionated into barium-soluble and barium-insoluble fractions by adding $BaCl_2$ at pH 8.2.

3. The barium-soluble supernatant was further divided into alcohol-soluble and alcohol-insoluble fractions by adjusting the concentration of ethanol to 80%. All three fractions were converted to sodium salts for assay in tissue culture.

4. Supplementation experiments indicate that for the assay system used the active growth factors in the crude dialysate are barium-soluble in aqueous solution and can be divided into two groups by differential solubility of the barium salts in 80% ethanol.

5. Both alcohol-soluble and alcohol-insoluble fractions are required to duplicate the growth-promoting effect of crude dialysate in a plasma-serum assay medium. In assay media containing dialyzed embryo extract the alcohol-insoluble fraction is dispensable.

6. The presence of glutathione selectively in the alcohol-insoluble fraction was demonstrated by paper chromatography. Assays in culture suggest that glutathione or cystine may account for a major part of the activity associated with the alcohol-insoluble fraction.

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THE RECIPROCAL NATURE OF GROWTH AND BEHAVIOR IN THE FETUS AND INFANT

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The intent of this paper is to illustrate a hypothesis of reciprocal relationship between somatic (cellular) growth and integrative (functional) growth in the human body. The illustration is most striking during the rapid growth phases of which I shall draw upon the consecutive periods of prenatal development and infancy for the accompanying graph.

SOURCE MATERIAL

The included graph represents a composite of data from various available sources: A. C. Wetzel ('32, '33),[1] for the growth rate in kg/yr, with the exception of one and one-half fetal months, for which the present author has calculated the growth rate, using the weight data of G. L. Streeter ('20, table 7); D. Hooker ('52, chap. 2) for the time of appearance and establishment of prenatal reflexes; and A. Gesell ('40, chap. 6, 8) for the development of postnatal activity.

DESCRIPTION OF GROWTH CHART

Somatic growth rate in terms of kg/yr [dz/dt] is charted along the ordinate as against critical prenatal and postnatal time intervals along the abscissa. It is evident from the Table that the most rapid increment of absolute somatic growth in terms of kg/yr obtains at birth. Enlarging upon this, I am including as the most rapid period of absolute growth the time during which the growth rate is at least 2/3 of the maximum at birth, i.e., the last three months of the prenatal period and the first six months of infancy. This rapid growth period is represented as the stippled area on the graph.

[1]Wetzel used Streeter's data for his prenatal calculations of kg/yr [dz/dt]; and a host of other sources for postnatal calculations: Gray and Ayres, Camerer, Czerny-Keller, Pirquet, Woodbury, Baldwin, and Boas.

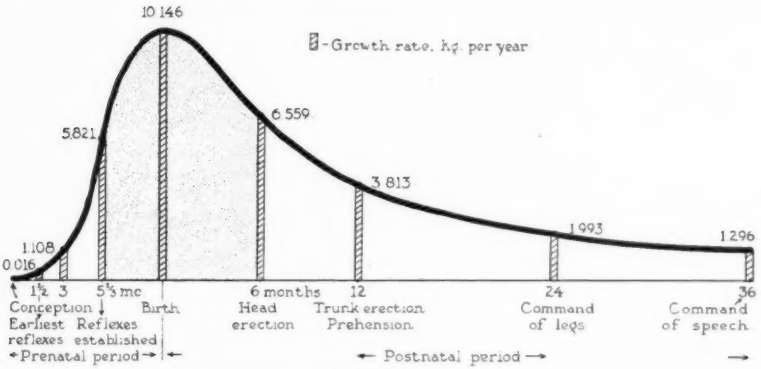

FIGURE 1

The reciprocal relationship between somatic growth in kg/yr and integrative growth, i.e. activity, as compiled by the author from the data of Streeter, Wetzel, Hooker and Gesell. The stippled area represents the period of most rapid somatic growth when progress in integrative growth is minimal. Fetal reflexes are established between 1 1/2 and 5 1/3 actual fetal months at which time somatic growth rate is slowly accelerating; integrated activity during infancy is developed after the first six months while the growth rate is rapidly decelerating.

During this maximal somatic growth phase, there is a minimum of integrative growth or activity in progress; the fetal reflexes are already established, as may be seen from the graph at five and one-third actual fetal months, and continue as such into early infancy.[1] In fact, the fetus at six months is already viable,[2] having the same repertoire of grasping, sucking, Moro, tonic neck, etc. reflexes as the full-term newborn and young infant.

By the time that the postnatal growth rate decreases to 2/3 of its neonatal maximum, the infant of six months has developed control of its head position. While the growth rate is only 1/3 of the neonatal maximum at twelve months, there has been rapid acceleration in the development of control of trunk stability as well as arm movements and prehension during the second half of the first year of life.

When the growth rate has diminished to 1/5 of the maximum at birth, the infant has gained command of his legs both as pillars and

[1]Fetal age is represented on the graph as "actual fetal" rather than as "menstrual"; and it is measured in Gregorian calendar months.

[2]According to Hooker ('52) some obstetricians have recorded fetuses of 25 weeks as viable, although the youngest viable fetus in his series was 27 weeks menstrual age. Moreover, he was able to demonstrate that few new fetal responses appear after 27 weeks menstrual age.

as locomotors: this occurs by the age of two years. Between the ages of two to three years, the child acquires the use of the fundamental structures of English, such as the noun-verb construction, tense discrimination and essential features of word order, along with an extensive working vocabulary: these, the ultimate tools of communication. From this point onward the decrement of growth is small; the lowest point is reached about the ages of three to four years, during which time the minimum rate of growth reached is 1/7 of the neonatal maximum.

Discussion of the Anthropometric Measurement of Choice

Growth in terms of the weight[3] increment is chosen for charting because it best represents three-dimensional growth of the body during its most rapid growth phase. Surface area data (E. Boyd, '41, plate 17; and '35) might have been selected because they represent a measurement in three dimensions, although these three dimensions are reduced to a two dimensional scale. Body growth expressed as height or as horizontal body length represents only one dimension of total body growth; and although it does fit the curve for three-dimensional growth during the postnatal infancy period (E. Boyd, '41, plate 17), it reaches its maximal increment not at birth but at three and one-third actual fetal months (G. L. Streeter, '20, table 1), thus coinciding with the period of the establishment of fetal reflexes. So that in the case of prenatal height increments, the relationship with integrative activity is more a direct rather than an inverse one.

Projected Theory of a Closed System of Energies

The above illustrated hypothesis of the inverse relationship between somatic body growth and integrative growth of activity in the human (and possibly in all other living creatures having periods of rapid growth and spurts of activity) suggests to me that the total energies available to the human body at any one time must comprise a closed system. And that for these available energies, somatic growth (cellular increase) and integrative growth (activity) must compete. This seems a reasonable conclusion from the fact that during the

[3]Simmons and Todd ('38) prefer to use height rather than weight as a measure of growth, especially following the second year of life and they cite Hammett ('36, '37) for substantiation. Hammett divides the weight increment into three definite, but indistinguishable components: not only an increase in growth of organs and supporting tissues, but an accumulation of fat as well as periodic fluctuations in degree of hydration.

periods of rapid somatic growth, such as the three or six months immediately preceding or following birth, respectively, there will be little further development of integrative activity; conversely, as the rate of somatic growth decreases, there are more energies made available for the advance of integrative growth as demonstrated during the early fetal months from one and one-half to five and one-third actual fetal months, with the origin and establishment of fetal reflexes along with viability of the creature; and again during infancy and early childhood after the first four to six postnatal months, followed by a steady succession of new activities.

Definition of Infancy

Since there has been some arbitrariness in the customary usages of the term infancy, e.g. the question whether one should include the first postnatal year (with or without the newborn period of two to three weeks) or the first two postnatal years, it seems advisable to go one step farther so as to include the first three years of postnatal life. The reason for the inclusion of the third year within the scope of infancy is so that no small portion of the rapid growth period should be omitted, that is up to the time of the minimum or leveling off of the growth rate, between three or four years; likewise by three years, the integrative growth has accelerated to the irreducible minimal level of control of bodily activity, including prehension, locomotion, the language communication. By three years, the child has the minimal requirement of body tools with which to build during the so-called preschool period, from three to six years. In fact, I should like to see a more descriptive term used for the preschool period, such as might indicate that the child is not only equipped with the basic tools to learn, but can already make use of them, in the learning process.

Summary

This paper illustrates the reciprocal relationship in humans (if not in other living creatures having rapid growth spurts) between somatic (cellular) growth represented by kg/yr, and integrative growth (activity), by compiling the available anthropometric measurements of A. C. Wetzel and G. L. Streeter, with the behavior data of D. Hooker and A. Gesell. The relationship is most strikingly evident by observation of the rapid growth periods and the author has chosen

the time of the fetus and the infant for purposes of charting. To the author's knowledge, this relationship has not previously been demonstrated.

A theory of distribution and competition for available body energies has been proposed as a likely setting for the succession of body growth which explains the necessity for an inverse proportion between somatic and integrative growth.

A more complete definition of the period of infancy is suggested from analysis of the materials.

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PERSISTENCE OF C^{14} IN A CALLUS CULTURE OF *SEQUOIA SEMPERVIRENS*[1]

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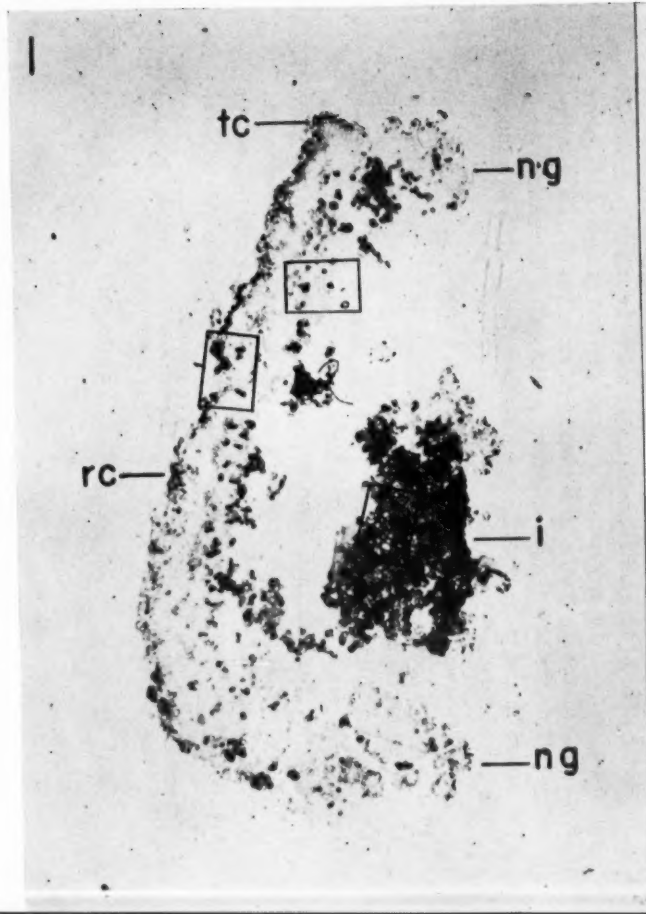
Since photosynthetic plants are usually entirely autophytic, the usual ways of introducing radioactive carbon into them are as $C^{14}O_2$, $NaHC^{14}O_3$, or as acetate. The use of a callus culture having heterophytic nutrition, however, makes possible the complete dependence of the growth of the plant tissues upon sugars in the medium. The earlier finding (Ball, 1953b) that sucrose supplied in the medium in the amount of 1.5 μc/ml. had no serious effect upon the growth of this callus made the latter suitable for studies on the retention of the radioisotope. The long half-life of C^{14} makes possible a long-term observation on such retention during subsequent transfers and growth upon non-radioactive medium. There is an important question about the metabolism of this callus culture that might be partially answered by such studies. Do organic substances (carbon compounds) move from the old tissues to the new ones? A variation of this question might be: do the new tissues produced by the callus live entirely from the sugars in the culture medium, or do they derive some substances from the old tissues?

Materials and Methods.—Cultures that had been started in 1951 (Ball, 1953a) on medium containing 1.5 μc of radioactive sucrose (uniformly labeled) per milliliter were continued by transferring them to non-radioactive medium. The ideal method would have been to grow the original culture indefinitely without subdivision upon reinoculation. The practical consideration, however, of limited space within a test tube made it necessary to cut up the culture before reinoculation so that the volume of tissue after two months of growth did not become excessively large. The procedure was as follows:

[1]Contribution from the North Carolina Agricultural Experiment Station. Published with the approval of the Director as Paper No. 524 of the Journal Series.


1
tc
ng
rc
i
ng

2
tc
tr

FIGURE 1

Unstained autoradiograph of callus grown two months on non-radioactive medium after initial 2.5-month exposure to C^{14}-sucrose-medium. The inoculum (i) had still a very great amount of the C^{14} as judged by the mass of silver grains in the emulsion of the NTB plate. The new growth (ng marks the positions of the marginal meristems) had only a few groups of cells with sufficient C^{14} to precipitate silver grains in the emulsion. By comparing with the next figure, it can be seen that the transported C^{14}-compounds were not stored in the marginal meristems nor in the cambia around tracheid groups. Some of the dark cells or groups of dark cells in the new growth are shown photographically because of their tannin content. The following labels may serve as a guide: (tc) tannin-containing cells near top surface of callus; (rc) radioactive group of cells that contained sufficient C^{14} to make a precipitation of silver grains in the emulsion. The C^{14} was obviously transported into the new growth from the inoculum. 10x.

FIGURE 2

Stained section, adjacent to the autoradiograph of Fig. 1, mounted on an ordinary glass slide. By comparing the location of tannin-containing (tc) cells, it is possible to determine which groups of cells of Fig. 1 are radioactive from translocated C^{14}-compounds. Tracheid group surrounded by cambium (tr) had, as can be seen in comparing with Fig. 1, insufficient C^{14} to make an autoradiograph. 10x.

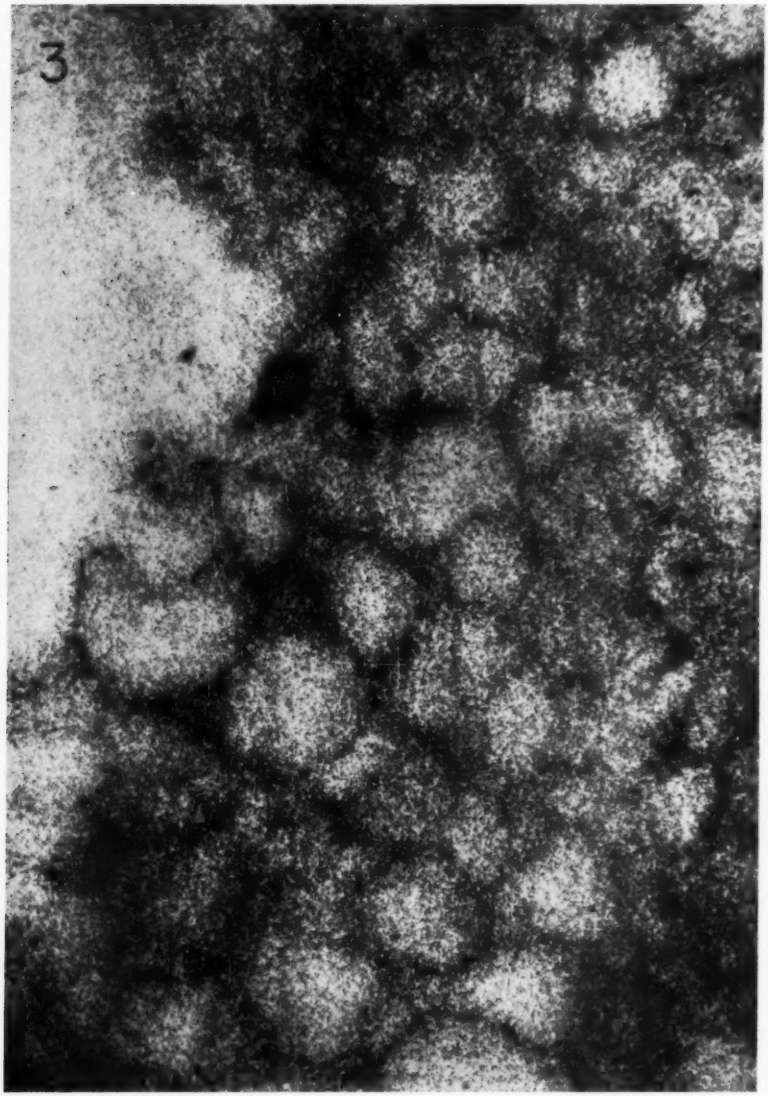


FIGURE 3

Magnified view of autoradiograph of inoculum of Fig. 1 (right rectangle) showing the very great number of silver grains in the emulsion of the NTB plate. Since most of the silver grains were below the peripheries of the cells, it may be assumed that most of the C^{14}-compounds were in the cytoplasm and walls of the cells of the inoculum at this time—two months after the callus had been taken from the radioactive medium. 196x.

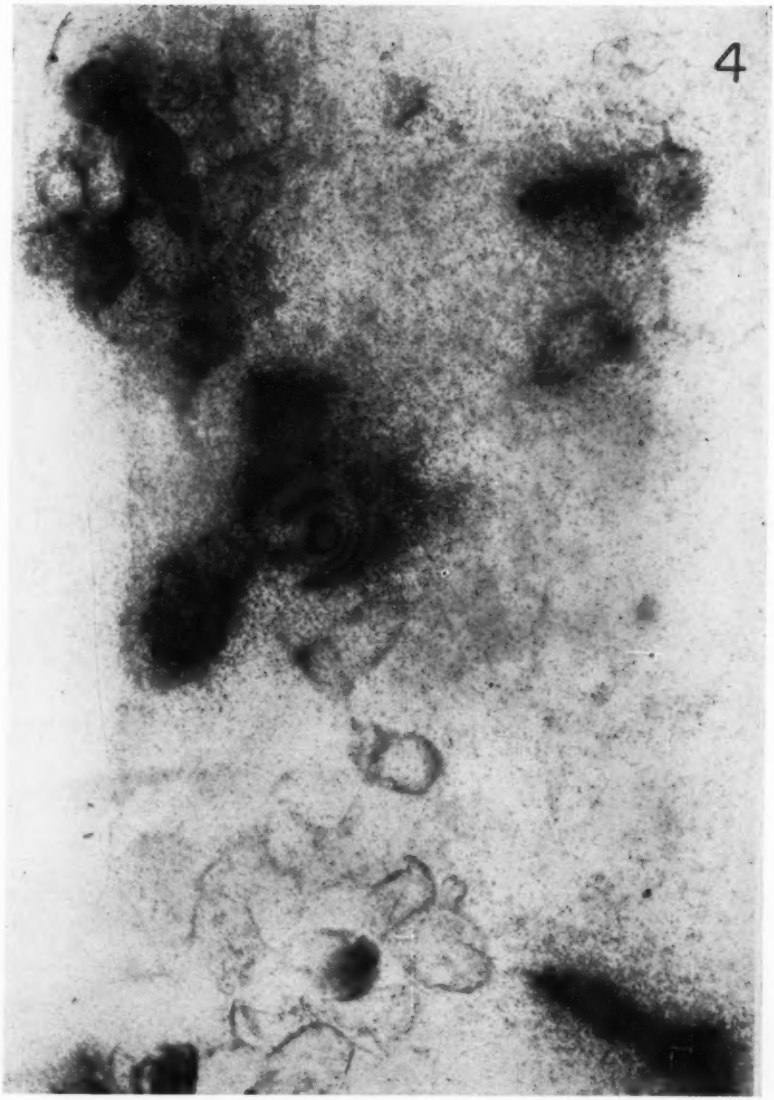


FIGURE 4

Magnified view of autoradiograph of groups of radioactive cells of the top surface of the new growth of Fig. 1 (left rectangle). The C^{14}-compounds that had caused the intense radioactivity in these groups of cells had apparently been transported from the inoculum. The silver grains in the emulsion indicate a uniform distribution of C^{14} throughout the cells; there was no concentration of radioisotope in the peripheries of the cells. Between these groups of highly radioactive cells the parenchymatous cells had a much lower content of C^{14}. 196x.

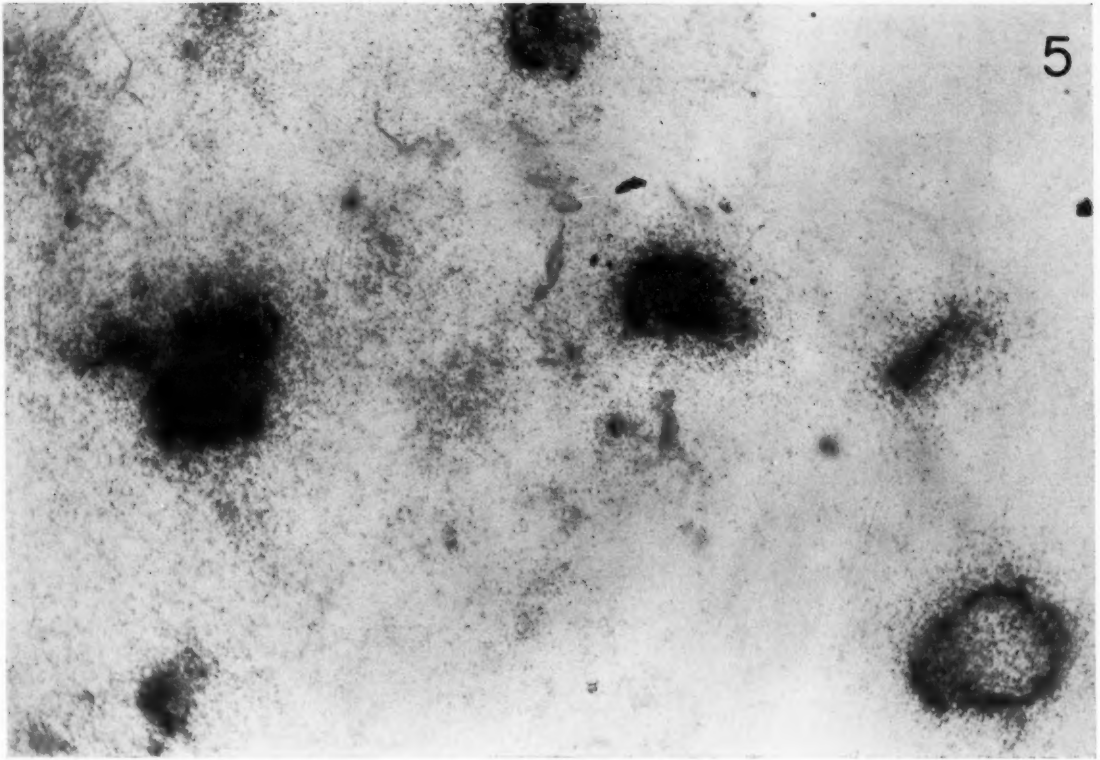


FIGURE 5

Magnified view of autoradiograph of internal cells of new growth of Fig. 1 (upper rectangle). Individual cells, or groups of cells contained much C^{14}, as judged by their autoradiographs, while intervening parenchymatous cells contained far less of it. There was no concentration of radioisotope in the peripheries of the cells. An equal distribution of silver grains over the cell indicated a uniform distribution of it. 196x.

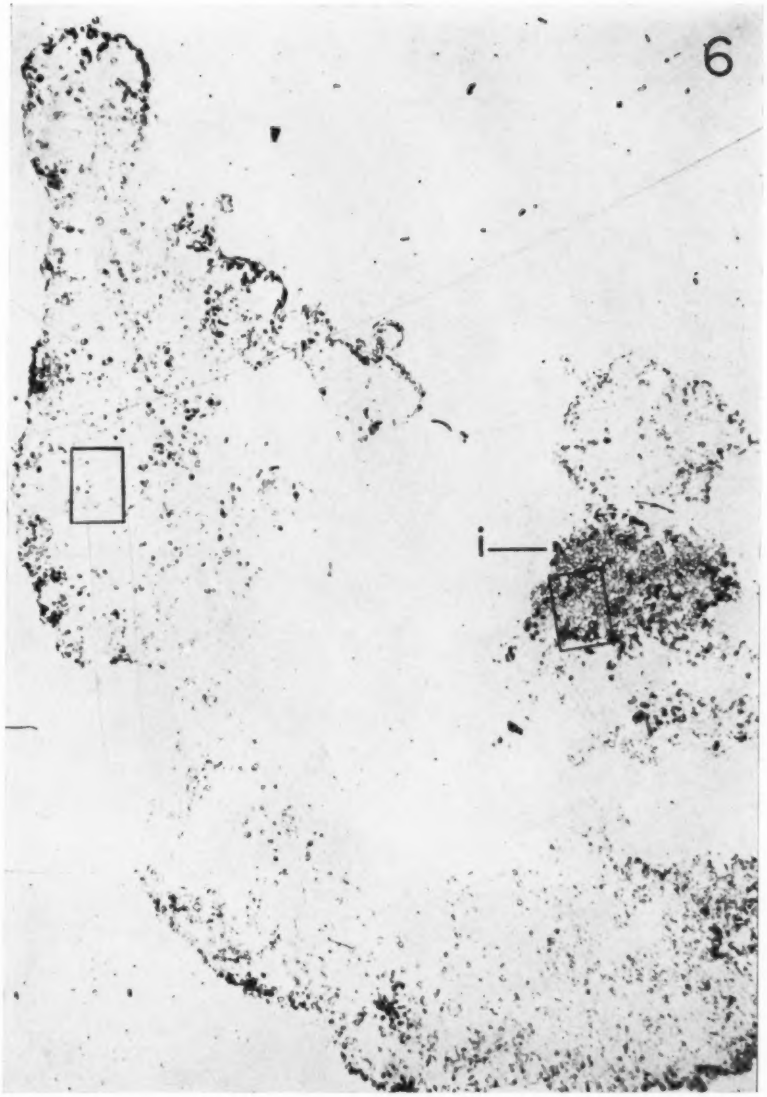


FIGURE 6

Autoradiograph of callus grown ten months (five transfers) on non-radioactive medium after initial 2.5-month exposure to C^{14}-medium. The older part of the inoculum (i) still had a considerable amount of C^{14} in it, as judged by the number of silver grains precipitated in the emulsion of the NTB plate. The amount of C^{14} in the new growth was correspondingly small and did not show at this low magnification. 10x.

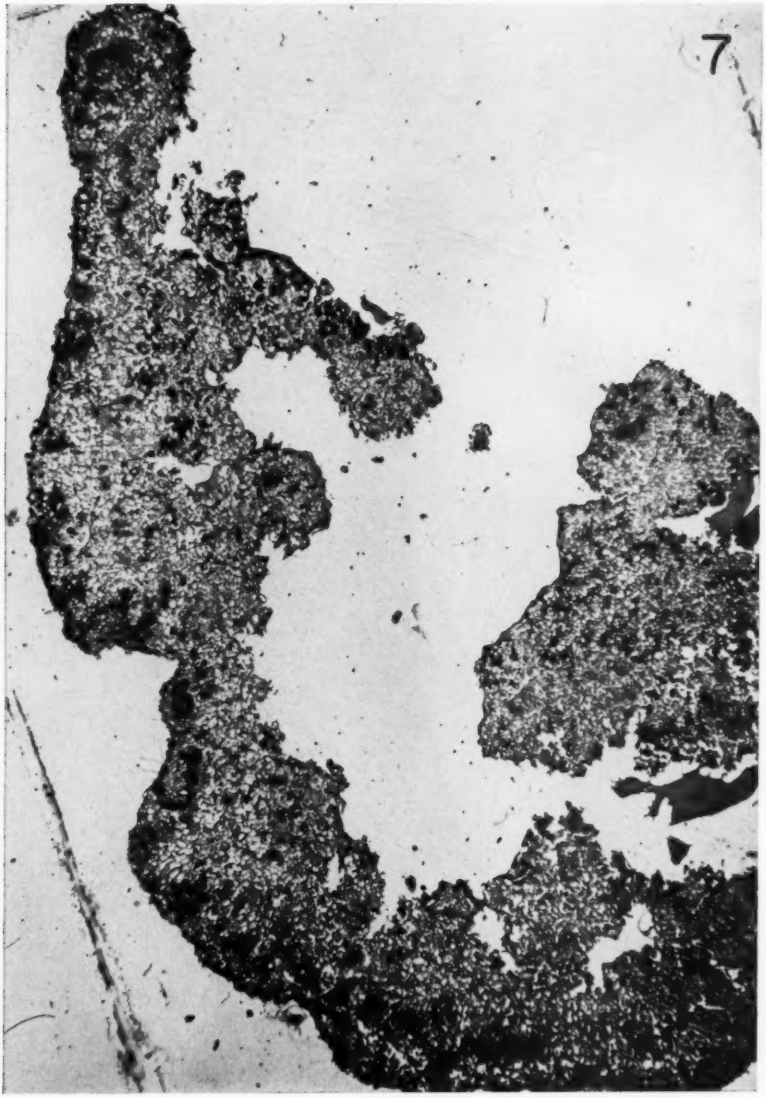


FIGURE 7

Stained section, adjacent to the autoradiograph of Fig. 6, mounted on an ordinary glass slide. 10x.

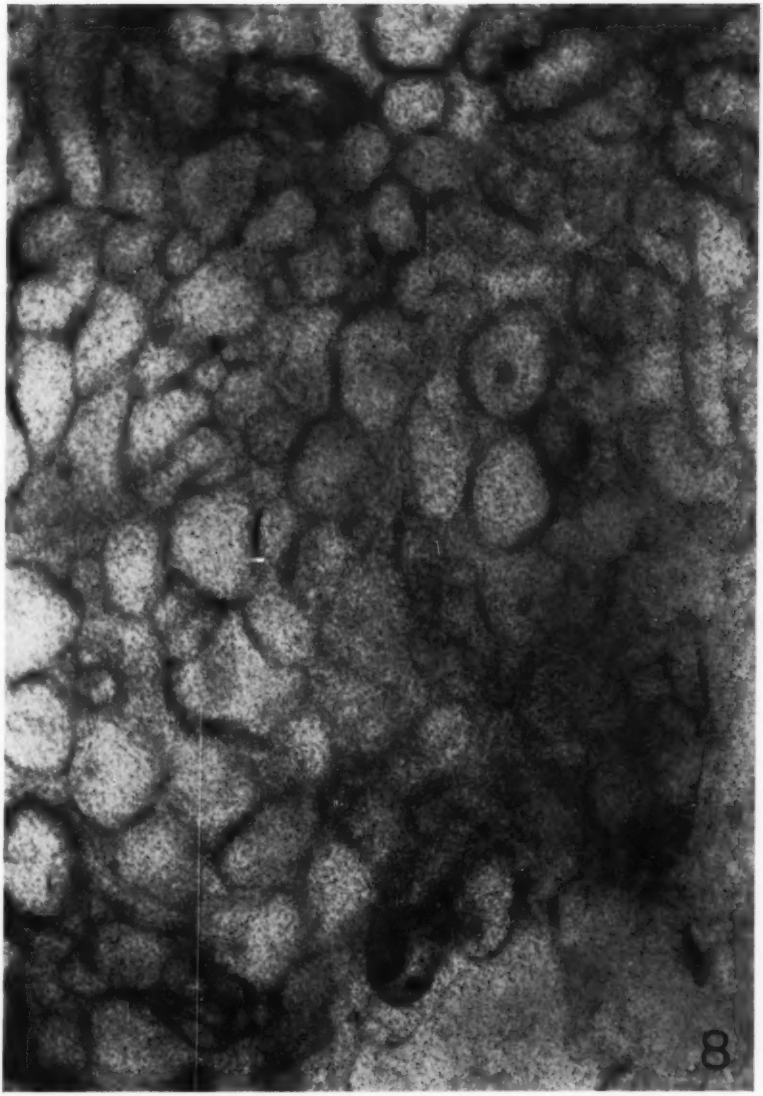


FIGURE 8

Magnified view of the old, highly radioactive part of the inoculum, showing the great number of silver grains in the emulsion of the NTB plate (right rectangle of Fig. 6). In most of the cells the greatest amount of the radioisotope was in the cytoplasm and cell walls. Although these cells were still highly radioactive, the amount of C^{14} had obviously decreased very much during the 8-month interval since the stage shown in Fig. 3. 196x.

FIGURE 9

Magnified view of autoradiograph of group of radioactive cells near margin of new growth of Fig. 6 (left rectangle). At this stage, after more than ten months of growth on non-radioactive medium, only small amounts of C^{14}-containing substances were transported from the old tissue to the new growth. The group of radioactive cells had only sufficient C^{14} to make a weak autoradiograph while the wide expanses of cells between such groups had no radioactivity at all, as judged by the absence of silver grains in the medium. There was equal distribution of silver grains over each cell of these radioactive cell groups, indicating no concentration of radioisotope in cytoplasm and cell wall. 196x.

after the initial exposure of the cultures by growing them on the radioactive medium for approximately two and a half months, the cultures were cut into fourths and inoculated onto ordinary Knop's medium with 3% sucrose. After two months of growth on this nonradioactive medium, the cultures were removed, cut into fourths, and again inoculated onto new nonradioactive medium. These reinoculations were continued through five transfers. The last transfer utilized in this study was approximately eight months after the original exposure of the cultures to the radioisotope. At the end of each growth period samples of the cultures that had grown upon the ordinary medium were removed from the tubes, killed, dehydrated, embedded in paraffin and sectioned according to procedures described earlier (Ball, 1953a). It was found that the paraffin ribbons were decreasingly radioactive from the first through the fifth growth period by measurements of counts with a Geiger-Müller tube held 1.5 mm. from them. Since the radioactivity of the ribbons grew progressively weaker from the first through the fifth transfer, it was decided to make separate stained preparations from the autoradiographs. Accordingly, the ribbons, cut at 15μ, were each divided into twenty portions suitable to be mounted under 60 mm.-long coverglasses. Alternate ones were mounted with adhesive on ordinary glass slides and were stained histologically. The portions between the latter were mounted on NTB plates (emulsion thickness 10μ) according to the method outlined by Ball (1953a). Since staining might have interfered with the photography of the silver grains in the emulsion, the autoradiographs were left unstained. The unstained autoradiographs were especially helpful for calluses in the fifth transfer where only a small number of silver grains was in the emulsion.

The exposure time was thirty days. Cover slips were mounted over Harleco Synthetic resin on the autoradiographs and adjacent stained preparations. Comparisons were made photographically between the autoradiographs and the next section that had been mounted on a glass slide and stained histologically.

Results and Discussion.—Since the callus grows outward by marginal meristems over the surface of the agar (Ball, 1950) the procedure of dividing the culture into four inocula usually carried over some of the original radioactive tissue in the second through the fifth transfers. The new growth was easily distinguished from the old in the autoradiographs. By the fifth transfer, at which time the radio-

activity of the cultures was only a fraction of that in the beginning, in most cases none of the original radioactive tissue was carried over in the inoculum. The autoradiographs of Figs. 6, 8, 9 were made of a callus that showed exceptionally high radioactivity at the fifth transfer, an indication that some of the original tissue was included.

Autoradiographs showed that the C^{14} in the original tissue remained during ten months of growth although subject to a progressive decrease in amount. There was progressive decrease in counts from the G.-M. tube held both against the calluses and the paraffin ribbons from them from the first through the fifth transfers. There was a much decreased number of silver grains in the emulsion under sections made after the fifth transfer (cf. Figs. 1 with 6 and 3 with 8). It is assumed that this decrease, although most of the C^{14} in the old tissues appeared to be in the cytoplasm and cell walls (Figs. 3, 8), was due to metabolism and included both respiration and transport of C^{14}-compounds. The original tissue appeared to be living, since the cells retained nuclei and cytoplasm in all histological preparations that were made. A physiological basis is thus present for the decrease in and transport from of C^{14}-compounds in this tissue.

The transport of C^{14}-compounds from the original tissues to new tissues is a revealing manifestation of the metabolism of this callus culture. It gives a partial answer to the question: does the callus live entirely on the sugar currently provided in the medium, or does it also utilize stored substances in the inoculum? Earlier work (Ball, 1953a) demonstrated that the callus utilized C^{14}-sucrose provided in the medium, since almost every cell of the callus made a more or less strong autoradiograph. The present work demonstrates that there was movement of C^{14}-compounds into new tissues during ten months of growth (five transfers). The location of the C^{14}-compounds in the new growth provided some interest and also raises some questions. Single parenchymatous cells, or often groups of them in the new growth showed a high content of C^{14}-compounds. Intervening parenchymatous cells showed a much lower content of C^{14} during the first transfer (Figs. 4, 5) and hardly any of the radioisotope during the fifth transfer (Fig. 9). Why did these single cells, or small groups of cells, accumulate the C^{14}-compounds that had been transported from old tissues? These accumulating cells were often, but not always, parts of the top surface of the callus. They were never parts of the marginal meristems (ng of Fig. 1) and never parts of the cambia

around tracheid groups. Furthermore, they were never parts of the phloem-like tissue that often had been produced externally by the cambia around the tracheid groups. The putative vascular tissue of these tracheid groups thus appeared to have no function in transport of the C^{14}-compounds. There was a further difference in the manner of accumulation of C^{14}-compounds in the new tissues. The silver grains were not most numerous at the periphery of the cells, but were uniformly distributed over the cell (Figs. 4, 5, 9). There were thus striking differences in manner of accumulation of C^{14}-compounds between a callus grown on radioactive sucrose and one already having C^{14}-compounds in its old tissues. In the former case the greatest accumulations were in the cambial cells around tracheid groups and in the marginal meristems (Ball, 1953a). These differences support the interpretation that radioactive sucrose absorbed from the medium is metabolized differently from the C^{14}-compounds translocated from old tissues.

The finding of Clendenning and Gorham (1952) that C^{14} was incorporated into the cytoplasmic proteins and cell walls of aquatic plants raises the possibility that such fixed radioactive carbon might be later removed and translocated to other parts of the plant. Another comparison to the present work is the formation of C^{14}-labeled ovule hairs in cotton by Greathouse (1953). He found that about 44% of the C^{14} that was introduced into the cotton boll by splitting its stem and placing the cut end in a solution of radioactive sugar could be found in the hydrolyzed cellulose. Since cotton ovule hairs lose their protoplasts at maturity and the fruit is generally taken from the plant, these experiments cannot indicate whether the C^{14} thus fixed could be translocated into new growth by the same plant.

Long-term retention of C^{14} in the bones of experimental animals has been reported (Bloom, Curtis and McLean, 1950; Skipper, Nolan and Simpson, 1951). In the latter case the C^{14} in the bone was utilized in metabolism for the percentage of the radioisotope injected that was retained in the skeleton decreased from 0.25% at 24 hours to 0.01% at one year.

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ROENTGENOGRAPHIC RECORD OF SKELETAL GROWTH IN RELATION TO AGE AND BODY WEIGHT OF THE RABBIT; CALCANEUS AND TIBIA

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Although a number of quantitative, cross sectional studies on postnatal growth of the rabbit skeleton have been published, serial roentgenographic studies similar to those carried out on the human pelvis by Reynolds (1945, 1947), on the monkey skull by Gans and Sarnat (1951) and on the rat skull by Spence (1940) seem not to have been reported. However, Selman and Sarnat (1953) have figured serial roentgenograms of a single rabbit skull at 6, 10 and 16 weeks of age.

The present paper based on serial roentgenograms, will report increase in actual length of the calcaneus and tibia of unoperated, control extremities of the rabbit in relation to age and body weight.

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Materials and Methods

The animals and the experimental conditions of this study are the same as those of the study of tendon increase reported by Lowrance (1953). The results of the previous study, however, are stated chiefly in terms of relative values of experimental extremities. The present paper considers the actual bone lengths of control extremities and therefore supplements the study on tendon growth. In addition, it reports increase in mean body weight.

The total x-ray exposure of 0.75 roentgen (Lowrance, 1953) applied over the period 15 to 204 days is less than one one-thousandth that reported to produce retardation of postnatal skeletal growth

(Cole, 1945). Minimum surgical trauma due to implanting stainless steel markers in the tendo calcaneus of the extremities opposite the ones studied here involved neither periosteum (Wu and Miltner, 1937) nor bone (Pease, 1952), and did not affect the regular trends of increasing body weight and bone lengths of operated animals. Experimental, operated extremities did not differ significantly in actual length from control extremities at any time during growth (Table 2). Moreover, transverse lines similar to those reported in roentgenograms of children by Eliot, Souther and Park (1927), Todd (1937) and Sontag (1938) are absent in roentgenograms of this series of rabbits. It is assumed, therefore, that the present heterogeneous group of rabbits shows an essentially normal pattern of skeletal growth.

In this study tibia length is the maximum distance from the intercondyloid eminence to the lateral malleolus (Bensley, 1938). Calcaneus length is the maximum length of that bone. Lengths are reported in film distances corrected for magnified radiographic distortion (Files, 1949), and were determined with a vernier caliper gradu-

TABLE 1

Mean body weights, significant ratios and coefficients of variation in relation to age.

AGE IN DAYS	FEMALE	MALE	SIGNIFICANT RATIO (S.R.) BETWEEN MALE AND FEMALE	MALE AND FEMALE COMBINED	
	MEAN	MEAN		MEAN	COEFFICIENT OF VARIATION
	GMS.	GMS.		GMS.	
15	235.5± 13.0	212.3± 16.2	1.11	222.4 ± 10.5	39.7 ± 3.85
22	345.8± 21.7	316.0± 25.1	0.89	329.5 ± 16.4	41.2 ± 4.08
29	546.1± 32.2	495.9± 30.1	1.14	518.8 ± 21.5	35.3 ± 3.28
36	766.8± 41.1	679.1 ± 33.3	1.66	719.0 ± 25.8	30.6 ± 2.78
43	951.1± 54.3	900.0± 40.5	0.75	923.2 ± 32.1	29.7 ± 2.67
50	1156.4± 63.7	1083.3 ± 40.3	0.97	1116.6 ± 35.4	27.0 ± 2.40
57	1350.3± 68.0	1261.3 ± 42.6	1.11	1301.8 ± 37.8	24.7 ± 2.17
64	1524.8± 71.5	1486.1 ± 52.0	0.44	1503.7 ± 41.8	23.7 ± 2.07
71	1709.6± 75.0	1661.7 ± 56.6	0.51	1683.5 ± 44.7	22.6 ± 1.97
78	1842.4± 68.0	1831.7 ± 53.3	0.12	1836.6 ± 41.1	19.1 ± 1.64
85	1958.0± 73.3	1970.0 ± 60.5	0.13	1964.6 ± 45.5	19.7 ± 1.70
92	2122.4± 83.5	2109.0 ± 70.1	0.12	2113.3 ± 52.0	21.0 ± 1.82
106	2326.7± 92.8	2301.2 ± 74.4	0.21	2312.8 ± 56.4	20.8 ± 1.80
120	2493.3± 94.0	2508.1 ± 84.7	0.12	2501.4 ± 61.0	20.8 ± 1.80
134	2637.0± 98.2	2623.9 ± 89.2	0.10	2629.9 ± 64.0	20.7 ± 1.79
148	2751.1± 103.1	2753.7 ± 97.2	0.02	2752.6 ± 68.6	21.2 ± 1.84
162	2837.0± 99.7	2866.6 ± 95.0	0.21	2853.2 ± 66.8	19.9 ± 1.72
176	2908.0± 102.8	2931.5 ±104.8	0.16	2920.9 ± 68.9	20.1 ± 1.73
190	2951.2± 101.6	3058.7 ±108.0	0.72	3009.9 ± 72.7	20.6 ± 1.78
204	2977.6± 100.7	3048.6 ±104.6	0.45	3013.0 ± 71.0	20.1 ± 1.71

ated to 0.1 mm. placed with the back parallel to the long axis of the bone image on the x-ray film. Weights were determined with a balance reading in whole grams.

Twenty arrays of mean measurements based on a fixed time schedule (Lowrance, 1953), between 15 and 204 days after birth for each of 33 rabbits are reported for body weight, and for lengths of calcaneus and tibia. In addition, individual actual length values of calcaneus and tibia were regrouped in class intervals for each 200 grams increase in body weight, and 19 separate arrays of measurements were organized.

Statistical constants, determined separately for members of each array, include the mean and its probable error, the standard deviation, the coefficient of variation and its probable error (Mainland, 1938). Significant ratio (S.R.) or difference between mean values was determined by a formula employed elsewhere (Lowrance, 1953). A difference (S.R.) of 3 or more is considered to be significant.

Values for male and female animals were determined separately but these were combined for final consideration.

Empirical formulae were developed to describe all increases reported in order to render the primary data useful in predicting growth of individual animals in other populations.

Results

Females tend to be slightly heavier than males between 15 and 134 days but after 134 days the reverse is true. None of the differences in weight is significant (Table 1).

Mean body weights of combined sexes at stated ages are listed in Table 1. If plotted, these mean body weights (Y) would show a smooth curve of increase in relation to time (X). In order to save space this curve is not figured in this paper, but it may be described in the following formulae:

$$Y = 115.5 - 0.238X + 0.475X^2 \quad (1)$$

between 15 and 36 days:

$$Y = 27.547X - 265.8 \quad (2)$$

between 36 and 71 days; and

$$Y = 29.1 + 28.573X - 0.0681X^2 \quad (3)$$

between 71 and 204 days.

Mean lengths of the calcaneus and tibia at stated ages are listed in Table 2. The following empirical formulae describe increase in length of the calcaneus (Y) on age (X):

$$Y = 4.493 + 0.6234X - 0.00459X^2 \quad (4)$$

between 15 and 71 days; and

$$Y = 25.81 + 0.01023X \quad (5)$$

between 71 and 204 days. The trend of increase in length of the tibia (Y) on age (X) between 15 and 162 days may be described by the empirical formula,

$$Y = 20.0 + 1.209X - 0.00419X^2 \quad (6)$$

and between 162 and 204 days by the formula,

$$Y = 100.98 + 0.0407X \quad (7)$$

Increase in mean length of calcaneus and tibia in relation to in-

TABLE 2

Mean lengths of calcaneus and tibia and their variation, and significant ratios between unoperated and operated extremities in relation to age.

AGE IN DAYS	LENGTH OF CALCANEUS			LENGTH OF TIBIA		
	MEAN	COEFFICIENT OF VARIATION	SIGNIFICANT RATIO (S.R.) BETWEEN CONTROL & OPERATED EXTREMITY	MEAN	COEFFICIENT OF VARIATION	SIGNIFICANT RATIO (S.R.) BETWEEN CONTROL & OPERATED EXTREMITY
	MM.			MM.		
15	12.7±0.21	13.83±1.19	0.34	35.5±0.43	10.21±0.86	0.16
22	15.9±0.23	10.58±1.04	0.00	43.2±0.55	9.26±0.91	0.13
29	18.7±0.18	10.04±0.99	0.56	51.1±0.59	8.41±0.82	0.37
36	21.5±0.22	8.89±0.74	0.32	59.3±0.59	8.29±0.70	0.12
43	23.0±0.23	8.59±0.72	0.00	65.7±0.58	7.58±0.63	0.00
50	23.9±0.25	8.84±0.75	0.56	71.0±0.61	7.15±0.61	0.12
57	24.8±0.23	7.75±0.67	0.00	76.2±0.60	6.64±0.56	0.00
64	25.3±0.26	8.55±0.73	0.27	80.9±0.58	6.02±0.51	0.00
71	26.0±0.28	9.13±0.78	0.25	85.1±0.63	6.14±0.53	0.00
78	26.5±0.29	9.20±0.78	0.25	89.1±0.55	5.14±0.43	0.13
85	26.8±0.29	8.81±0.76	0.00	91.9±0.57	5.26±0.44	0.13
92	26.8±0.29	8.99±0.76	0.00	95.3±0.57	5.09±0.42	0.12
106	27.0±0.29	9.23±0.77	0.25	99.4±0.61	5.15±0.44	0.24
120	27.3±0.31	9.32±0.82	0.23	103.2±0.72	5.76±0.49	0.10
134	27.6±0.29	8.58±0.75	0.00	105.7±0.66	5.05±0.44	0.30
148	27.3±0.29	9.05±0.76	0.25	106.8±0.67	5.13±0.45	0.20
162	27.5±0.30	8.95±0.77	0.00	107.8±0.72	5.51±0.47	0.30
176	27.7±0.32	9.17±0.83	0.00	108.3±0.77	5.74±0.50	0.10
190	27.6±0.28	8.49±0.73	0.25	108.5±0.65	4.83±0.42	0.09
204	27.6±0.29	8.85±0.74	0.00	109.3±0.70	5.43±0.45	0.20

crease in mean body weight (Table 3) is less regular than increase in length of these bones in relation to age, because weight itself is a variable. Between body weights (X) of 165 and 1287 grams, mean calcaneus length (Y) may be described by the formula,

$$Y = 8.023 + 0.02695X - 0.0000112X^2 \quad (8)$$

and by the formula,

$$Y = 21.06 + 0.002505X \quad (9)$$

between 880 and 3675 grams. The principal increase in the mean length of the tibia (Y) occurs between 165 and 686 grams mean body weight (X). The empirical formula describing this increase is,

$$Y = 23.43 + 0.06676X - 0.00001787X^2 \quad (10)$$

The equation which is valid between 686 and 1886 grams mean body weight may be stated,

$$Y = 42.02 + 0.02777X \quad (11)$$

and the formula which is applicable in the mean body weight interval 1696 to 3675 grams,

$$Y = 67.53 + 0.01326X \quad (12)$$

The average percentage deviations of the calculated values from the means of observed values in the above numbered formulae are as follows: (1), 1.85; (2), 0.49; (3), 0.73; (4), 1.09; (5), 0.64; (6), 1.43; (7), 0.15; (8), 1.43; (9), 1.07; (10), 0.47; (11), 0.38; (12), 0.75. All empirical formulae closely fit the means of those observed values falling within the intervals to which the formulae apply.

The coefficients of variation in mean body weight (Table 1) are highest in very young animals, but decrease progressively to approximately 20% after 78 days.

Variation in length of the calcaneus in relation to time (Table 2) is greatest in the very young animals since these have high rates of growth. It attains a relatively constant value of approximately 9% by 36 days. Variation in length of the tibia (Table 2) at its maximum is almost three-fourths that of the calcaneus. It decreases more gradually with time than does that of the calcaneus, reaches a relatively constant value of approximately 5% at 78 days or about the time when variation in body weight has become stabilized. Coefficients

TABLE 3

Mean lengths of calcaneus and tibia and their variation in relation to mean body weight.

MEAN BODY WEIGHT	LENGTH OF CALCANEUS		LENGTH OF TIBIA	
	MEAN	COEFFICIENT OF VARIATION	MEAN	COEFFICIENT OF VARIATION
GMS.	MM.		MM.	
164.7 ± 3.33	11.9 ±0.19	9.31 ±1.12	33.8 ±0.42	7.64 ±0.89
282.9 ± 5.53	14.9 ±0.19	12.09 ±0.91	41.2 ±0.51	11.82 ±0.89
501.9 ± 5.88	19.1 ±0.15	6.04 ±0.56	52.2 ±0.50	7.36 ±0.68
686.4 ± 7.23	21.3 ±0.19	7.09 ±0.62	60.9 ±0.53	6.88 ±0.61
879.6 ± 6.38	22.6 ±0.18	6.91 ±0.57	66.1 ±0.54	7.18 ±0.58
1091.0 ± 6.37	24.1 ±0.26	8.96 ±0.76	72.5 ±0.56	6.47 ±0.55
1286.8 ± 6.75	24.3 ±0.16	5.71 ±0.46	78.3 ±0.70	7.75 ±0.64
1487.1 ± 5.62	25.0 ±0.16	6.04 ±0.45	83.6 ±0.71	8.39 ±0.61
1695.9 ± 5.14	25.3 ±0.17	6.83 ±0.47	88.9 ±0.67	7.79 ±0.54
1885.8 ± 5.66	25.8 ±0.16	5.97 ±0.45	94.1 ±0.66	6.69 ±0.50
2088.9 ± 6.22	26.7 ±0.17	6.19 ±0.44	96.0 ±0.60	6.07 ±0.44
2294.7 ± 6.89	27.4 ±0.25	6.96 ±0.65	96.8 ±0.67	5.22 ±0.49
2492.5 ± 6.62	27.2 ±0.19	6.53 ±0.51	99.9 ±0.58	3.61 ±0.28
2696.5 ± 7.01	27.6 ±0.32	9.13 ±0.83	103.5 ±0.46	3.45 ±0.31
2889.3 ± 7.40	28.0 ±0.25	6.39 ±0.64	106.0 ±0.48	3.13 ±0.32
3133.0 ±13.20	28.3 ±0.14	4.61 ±0.35	109.1 ±0.33	2.89 ±0.22
3284.1 ± 5.60	29.3 ±0.19	5.24 ±0.45	112.1 ±0.42	3.00 ±0.27
3496.3 ± 8.82	29.5 ±0.25	6.10 ±0.61	112.7 ±0.62	3.19 ±0.39
3675.3 ±10.22	30.9 ±0.32	5.34 ±0.74	116.6 ±0.87	3.87 ±0.53

of variation of length of the calcaneus in relation to mean body weight fluctuate, those of the tibia tend to decrease with increasing mean body weight to a mean weight of 2493 grams (Table 3). Between mean body weights of 2493 and 3675 grams the coefficients of variation of tibia length average 3.3%.

All of the mean values and 93% of the coefficients of variation exceed their respective probable errors by a factor of 10 or more and therefore are considered to be reliable (Tables 1, 2, and 3).

Summary

Tables are presented which report mean body weight and its variation, and mean lengths and variability of calcaneus and tibia, of a heterogeneous population of rabbits serially x-rayed from 15 to 204 days after birth. Empirical formulae are offered to describe increases in mean body weight and bone lengths.

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BOOK REVIEW

Problems of Infancy and Childhood. Josiah Macy, Jr. Foundation, New York City, 160 pages, 1953.

REVIEWED BY WILLARD C. OLSON

The Josiah Macy, Jr. Foundation has sponsored a number of conference groups designed to promote meaningful communication among scientific disciplines. The conferences were set up in recognition of the fact that the continued isolation of the several branches of science is a serious obstacle to scientific progress.

As stated in a foreword by Frank Freemont-Smith, the Medical Director, the conferences are designed to increase the fertility of the multi-discipline approach. The meetings are contrasted to the usual scientific gatherings in that they do not attempt to present neat solutions to tidy problems but to elicit provocative discussion. The results are reported to make current thinking in a field available to all those working in it. The conference process is designed to promote creativity as well as logic. In an informal setting conferees are encouraged to speculate freely and to use intuitive or other approaches that may help in synthesis or in the setting of a problem for more rigorous testing.

The Transactions of the Sixth Conference on Problems of Infancy and Childhood contains short reports by Knut Kjellberg on "A Brief Review of WHO Activities", by Katherine M. Wolf on "Individual Tendencies in the First Year of Life", and by Ann Stewart on "Excessive Crying in Infants—A Family Disease."

The major part of the record is devoted to a paper by Sibylle Escalona of the Child Study Center of Yale University on the topic "Emotional Development in the First Year of Life". Dr. Escalona relates observations and interpretations growing out of studies of infants made at the time she was with the Menninger Foundation in Topeka. She adopts the role of a skilled observer diligently recording a great deal of factual material and at the same time willing to "feel with" and to try to understand the affective side of the process of interaction between mother and child.

The report provides much detail on the style of a mother with a

child and of the characteristics of different infants. This detail makes it clear why generalizations are difficult on the effects of early child rearing practices on later personality development. On the whole she emphasizes the reciprocal nature of the reaction between mother and child with the possibility of the reactions of the mother being in part attributable to difficulties emanating from the baby as well as from any anxieties or resentment of the maternal role on the part of the mother. She points out that children differ greatly in such matters as the amount of activity, the degree of impulsiveness, and on skin sensitivity. Mothers, for example, differ greatly in the extent to which they reduce physical contact with the baby to a minimum or to which they tickle, stroke, kiss, pat, and adjust almost continuously. In her discussion of interaction of the organism with the environment she points out that this can become very complex so that very different constellations of circumstances can result in very similar behavior.

She finds space, time, and reality among the dimensions of experience of importance in the first year of life. Contagion and communication are important processes in social learning.

The discussion among the participants in the conference is faithfully reported. Interruptions of the presentation occur in an informal way and breaks are provided at intervals. The discussants are all well known investigators and practitioners who work with children and who are able to bring out nuances of concept and important details of methods.

The report reveals much insight into the process or dynamics of growth and change and development of personality. The exhibit is also of special interest currently in showing a methodological change from the reconstructive methods which have dominated some recent approaches to child study to an attempt again at direct observation with an acknowledgment that observation and reconstruction may be complementary.

The report is primarily an exploration of the growing edge of an attempt to understand organized complexity.